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Antipyretic and antinociceptive effects of *Nauclea latifolia* root decoction and possible mechanisms of action

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Abstract

Context

Nauclea latifolia Smith (Rubiaceae) is a small tree, found in tropical areas in Africa. It is used in traditional medicine to treat malaria, epilepsy, anxiety, pain, fever etc.

Objective

The aim of this study was to investigate the effects of *Nauclea latifolia* roots decoction on the peripheral and central nervous systems and its possible mechanisms of action.

Materials and methods

The analgesic investigation was carried out against acetic acid-induced writhing, formalin-induced pain, hot-plate and tail immersion tests. The antipyretic activity was studied in Brewer's yeast-induced pyrexia in mice. Rota-rod test and bicuculline-induced hyperactivity were used for the assessment of locomotor activity.

Results

Nauclea latifolia induced hypothermia and had antipyretic effects in mice. The plant decoction produced significant antinociceptive activity in all analgesia animal models used. The antinociceptive effect exhibited by the decoction in the formalin test was reversed by the systemic administration of naloxone, N_ω-L-nitro-arginine methyl ester or glibenclamide. In contrast, theophylline did not reverse this effect. *Nauclea latifolia* (antinociceptive doses) did not exhibit significant effect on motor coordination of the mice in rota-rod performance. *Nauclea latifolia* protected mice against bicuculline-induced behavioural excitation.

Discussion and conclusion

Overall, these results demonstrate that the central and peripheral effects of *Nauclea latifolia* roots decoction might partially or wholly be due to the stimulation of peripheral opioid receptors through the action of the nitric oxide-cyclic GMP-ATP-sensitive K⁺ (NO/cGMP/ATP)-channel pathway and/or facilitation of the GABAergic transmission.

Author Keywords *Nauclea latifolia*; antipyretic; antinociceptive; mechanism

MESH Keywords Analgesics; administration & dosage; pharmacology; Animals; Antipyretics; administration & dosage; pharmacology; Disease Models, Animal; Female; Fever; drug therapy; Male; Medicine, African Traditional; Mice; Motor Activity; drug effects; Pain; drug therapy; Plant Extracts; administration & dosage; pharmacology; Plant Roots; Receptors, Opioid; drug effects; metabolism; Rubiaceae; chemistry

Introduction

Nauclea latifolia Smith (Rubiaceae) is a shrub or small spreading tree that is a widely distributed plant that grows in the north Cameroon and other African countries (Arbonnier 2000). It is found in the forest and fringe tropical forests. In Tupuri language, in Cameroon it is known as "Koumkouma". *Nauclea latifolia* roots decoction is one of such herbal preparations that have been used traditionally for treating different disease conditions. Medicinal uses vary from one traditional setting to another, its traditional uses include: fever, pain, dental caries, septic mouth, malaria, dysentery, diarrhea, and diseases of the central nervous system such as epilepsy (Arbonnier, 2000, Amos et al., 2005; Ngo Bum et al., 2009; Abbah et al., 2009). The root of *Nauclea latifolia* is the preferred part of the plant used in Cameroonian traditional medicine for treating pain and fever. This part is usually harvested, sun dried and pulverized to

obtain powder. About 100 g of the powdered material is macerated in 500 ml of water and boiled. The decoction obtained is administered orally at the dose range of 80–160 mg/kg.

The aqueous extract of leaves of the plant has been used as a remedy for diabetes in northern Nigeria (Gidado et al., 2005). The plant also has been reported to have antihypertensive and laxative activities (Akpanabiantu et al., 2005). Previous works have shown that the aqueous extract of the bark of *Nauclea latifolia* (freeze-dried extract) attenuated writhing episodes induced by acetic acid and increased the threshold for pain perception in the hot plate test in mice. The extract remarkably decreased both the acute and delayed phases of formaline-induced pain in rats and also caused a significant reduction in both yeast-induced pyrexia and egg albumin-induced edema in rats (Abbah et al., 2009). In the course of pharmacological studies, anticonvulsant, anxiolytic and sedative properties of *Nauclea latifolia* roots decoction (Ngo Bum et al., 2009) have already been reported from this laboratory. Phytochemical investigations of the bark and wood of *Nauclea latifolia* have reported the presence of naucleamides A–E, new monoterpene indole alkaloids from *Nauclea latifolia* (Shigemori et al., 2002). Unfortunately, none of these compounds have been tested for their pharmacological activities.

The present study was therefore carried out to confirm the veracity of the aforementioned traditional claims of *Nauclea latifolia* roots decoction usefulness. Thus, we investigated the effects of *Nauclea latifolia* roots decoction on the peripheral and central nervous systems by using several experimental models of fever and pain in mice. To examine the possible mechanisms of the decoction in the antinociception effects, we used naloxone (a non-selective opioid receptor antagonist), theophylline (a non selective adenosine receptor antagonist), glibenclamide (an ATP-sensitive K⁺ channel inhibitor) and N_ω-L-nitro-arginine methyl ester (L-NAME, a NO synthase inhibitor). Preliminary acute toxicity and phytochemical tests were also carried out to evaluate the secondary metabolites present and the safety of this widely used plant.

Materials and methods

Plant material

The roots of *Nauclea latifolia* used in this study were collected in the dry season (March 2008) from the National Park of Benoué (North-Cameroon). Botanical identification was performed at the National Herbarium, Yaounde. Voucher herbarium specimen No 20144/SRF/Cam has been deposited at the Yaoundé herbarium.

Preparation of the extracts

The bark of dried roots of *Nauclea latifolia* was ground. The powder (1 000 g) was macerated in 5 000 ml of distilled water for 1 h. This mixture was boiled for 20 min. After it cooled, the supernatant (decoction) was collected and filtered with Whatman No. 1 filter paper. After filtration, water was evaporated in a drying oven at 45°C and 81 g of a dark brown solid was obtained. The yield of the extraction was 8.1%. The plant extracts (8 g) were dissolved in 250 ml of distilled water. This was the stock solution for the pharmacological tests. The decoction, prepared 30 min to 1 h before its administration in mice, was administered orally (p.o.) 1 h before the pharmacological test. The following doses were used: 16, 40, 80, 160, and 320 mg/kg. Other doses were prepared and administered either orally or intraperitoneally in acute toxicity study. For each other dose used, we calculated the volume to be follows:

$$V(\text{ml}) = \frac{D(\text{g/kg}) \times P(\text{kg})}{C(\text{g/ml})}$$

Where D is dose used (g/kg body weight), P is body weight (kg), C is concentration of the extract (g/ml) and V is volume of extract (ml).

Drugs and chemicals

Acetylsalicylic acid (Aspirin®, Laboratoires 3M, France), glibenclamide (Doanil®, was from Sanofi-Avensis, Guildford, UK), formaline (BDH, Pool, England whilst), theophylline (Xanthium®, SMB, France), morphine sulphate, naloxone hydrochloride, N_ω-L-nitro-arginine methyl ester, brewer's yeast (Arkopharma, Carros, France), acetic acid, bicuculline (Sigma Aldrich Inc., St Louis, MO, USA), indomethacin (Indocid®, MerckSharp-DolmeChibret, France) and diazepam (Valium®, was from Roche, France). Chemicals were prepared in the form of suspensions using a few drops of Tween 80 and diluted with distilled water. All treatments were administered in a volume of 10 ml/kg mice body weight.

Animals

Adult male and female mice (*Mus musculus* Swiss; 20–25 g) were used throughout these studies. The animals were housed in standard cages at 25°C on a 12 h light-dark cycle. They were supplied with food and water *ad libitum*. The experiments were carried out in accordance with the National Ethical Committee Guidelines (reg N°. FWA-IRB00001954) and International (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996) for the care and used of laboratory animals. All efforts were made to minimize both the suffering and number of animal used.

Preliminary phytochemical test

Preliminary phytochemical properties of the decoction of the roots of *Nauclea latifolia* were tested using the following chemicals and reagents: flavonoids (NaCl and HCl), alkaloids with Mayer and Dragendoff's reagents, saponins (frothing test), tannins (FeCl₃), glycosides (NaCl₃ and Fehling's solution A and B), cardiac glycosides (Salkowski test), anthraquinones (Borntrager's reaction), phenols (FeCl₃ and K₃Fe(CN)), and lipids (filter paper) (Trease & Evans, 1983).

Acute toxicity studies

Mice of either sex were divided in groups of 10 animals. All animals had free access to tap water and food, except for short fasting period before oral administration of single doses of the decoction of the roots of *Nauclea latifolia*. The extract was administered by gavage at the doses of 1000, 2000, 4000, 8000, 10 000, 12 000 and 14 000 mg/kg or by the intraperitoneal route at the doses of 100, 250, 500, 1000, 2000, 4000 and 8000 mg/kg. The general behavior of mice was observed continuously for 1 h after the treatment and then intermittently for 4 h, and thereafter over a period of 24 h (Twaij et al., 1983). The mice were further observed for up to 14 days following treatment for any signs of toxicity and deaths, and the latency of death. Any adverse effects, such as hypoactivity, piloerection, salivation, and syncope, were evaluated immediately after administration of *Nauclea latifolia* extract. Also, anorexia and weight loss were observed and noted. The LD₅₀ value was determined according to the method of Litchfield and Wilcoxon (1949). Confirmatory test was carried out and the LD₅₀ was calculated from the graph of percentage (%) of mortality (converted to probit) against log-dose of the extract.

Antipyretic activity

Body temperature

Four groups of mice received orally various doses of the *Nauclea latifolia* roots decoction (16, 40, 80 and 160 mg/kg, p.o.) and one group received distilled water. Rectal temperature was recorded with an electronic thermometer at predetermined times in groups of mice before and after (0, 0.5, 1, 2, 3 and 24 h) the administration of either distilled water or *Nauclea latifolia* decoction. Pre-drug recording served as the reference point for the determination of the temperature change (Pal & Nag, 1999).

Antipyretic test

An initial rectal temperature was recorded by insertion of an electronic thermometer 2 cm deep into the rectum. This was recorded again 30 min after and an average taken. Fifteen percent suspension of Brewer's yeast in 0.9% saline solution was prepared. Pyrexia was induced by injecting 20 mg/kg of Brewer's yeast suspension subcutaneously in the back behind the nuchae of the neck. Following the injection, the site was massaged in order to spread the suspension beneath the skin. The room temperature was kept at 30°C. The animals were starved for 18 h but water made available *ad libitum*. The rectal temperature measurement was done again 18 h post-injection to record its rise. Only mice with body temperatures greater than 36.5°C were taken into the test. The animals received the *Nauclea latifolia* decoction (16, 40, 80 and 160 mg/kg, p.o.) and standard drug (acetylsalicylic acid 150 mg/kg, p.o.) orally and the measurements were taken 0.5–6 h post-dosing (Brune & Alpermann, 1983).

Antinociceptive activity

Acetic acid-induced abdominal constriction

The plant decoction (16, 40, 80 and 160 mg/kg, p.o.), acetylsalicylic acid (150 mg/kg, p.o.), morphine (5 mg/kg, s.c.), naloxone + *Nauclea latifolia* decoction (1 mg/kg, i.p. + 160 mg/kg, p.o.) and distilled water (p.o.) were administered 1 h prior to acetic acid treatment. One hour after oral administration of these substances, each animal was injected intraperitoneally with 0.6% acetic acid in a volume of 10 ml/kg body weight. After acetic acid injection, the number of stretching or writhing responses per animal was recorded during a subsequent 30 min after a latency period of 5 min and permitted to express the percentage of inhibition (Asongalem et al., 2004).

Formaline-induced nociception

The formalin test was carried out as described by Hunskaar and Hole (1987) and few modification. The negative control was treated with distilled water. The positive control received indomethacin (10 mg/kg, p.o.) and morphine (5 mg/kg, s.c.) two reference analgesic compound. Four groups of mice were treated with the extract of plant (16, 40, 80 and 160 mg/kg, p.o.). Pain was induced by injecting 0.05 ml of 2.5% formalin (40% formaldehyde) in distilled water in the subplantar of the right hindpaw. Mice were given *Nauclea latifolia* decoction (16, 40, 80, 160 mg/kg, p.o.), indomethacin (10 mg/kg), morphine (5 mg/kg, s.c.), and distilled water (p.o.) 1 h after injecting formalin. These mice were individually placed in a transparent Plexiglas cage (15 × 15 × 15 cm) observation chamber. The amount of time spent licking and biting the injected paw was indicative of pain and was recorded in 0–5 min (first phase) and 15–30 min (second phase). The four other groups were pre-treated with: naloxone (2 mg/kg, i.p.), theophylline (5 mg/kg, i.p.), glibenclamide (8 mg/kg, p.o.) and N^ω-L-nitro-arginine methyl ester (L-NAME, 10 mg/kg, i.p.). After 15 min (pre-treatment with naloxone, theophylline and glibenclamide) and 30 min (pre-treatment with L-NAME), the mice received the *Nauclea latifolia* decoction at the dose of 160 mg/kg. The nociceptive response to the formalin intraplantar injection was recorded 1 h after administration of extract.

Hot plate

The apparatus consisted of a water bath in which was placed, a metallic cylinder (diameter 14 cm and 10 cm high). Water bath temperature was set at $55 \pm 0.5^\circ\text{C}$ (Lanhers et al. 1991). Each mouse (six per group) acted as its own control. Prior to treatment, the reaction time of each mouse (liking of the forepaws or jumping response) was done 0 and 10 min interval. The average of the two readings was obtained as the initial reaction time. The reaction time following the administration of the decoction (16, 40, 80 and 160 mg/kg, p.o.), acetylsalicylic acid (150 mg/kg, p.o.), morphine (5 mg/kg, s.c.), naloxone + *Nauclea latifolia* decoction (1 mg/kg, i.p. + 160 mg/kg, p.o.) and distilled water (p.o.), was measured at 0.5, 1, 2, 3, 4, 5 and 6 h after a latency period of 30 min (Asongalem et al., 2004).

Tail immersion

The tail immersion test was carried out according to the method described by Viswanatha et al. (2006) . This involved immersing extreme 3 cm of the mice's tail in water bath containing water at a temperature of $55 \pm 0.5^\circ\text{C}$. Within a few second, the mice reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. The mice treated with *Nauclea latifolia* decoction (16, 40, 80 and 160 mg/kg, p.o.), naloxone + *Nauclea latifolia* decoction (1 mg/kg, i.p. + 160 mg/kg, p.o.), a standard drugs, acetylsalicylic acid (150 mg/kg, p.o.), morphine (5 mg/kg, s.c.) and distilled water (p.o.). The reaction time of mice was taken at intervals 15, 30 and 60 min after a latency period of 1 h following the administration of the decoction and drugs.

Studies of motor coordination (rota-rod test)

Motor performance was assessed as previously report (Pieretti et al., 1999) with a rota-rod apparatus, consisting of a bar with a diameter of 3.0 cm, subdivided into five compartments by a disk of 24 cm in a diameter. The bar rotated at a constant speed of 16 revolutions per min. A preliminary selection of mice was made on the day of experiment excluding those that did not remain on the rota-rod bar for two consecutive periods on 45 s each. The integrity of motor coordination was assessed on the basis of the number of falls from the rota-rod in 180 s. Selected animals were tested immediately at 0, 30, 60, 90 and 120 min after administration of *Nauclea latifolia* decoction (16, 40, 80, 160 and 320 mg/kg, p.o.), diazepam (1 mg/kg, i.p.) or distilled water (10 ml/kg, p.o.).

Antagonism to bicuculline-induced behavioural excitation

The method has been described previously (Ngo Bum et al., 2001). In brief, mice were injected i.p. with bicuculline 2 mg/kg, 1 h after the oral administration of *Nauclea latifolia* roots decoction (16, 40, 80 and 160 mg/kg, p.o.), diazepam (3 mg/kg, i.p.) and distilled water (p.o.). They were placed on by one in the center of the open field. The open field used was a wooden square box (40 cm × 40 cm × 45 cm) was devised into 16 smaller squares of equal dimensions (10 cm × 10 cm). Mice were observed 1 h. rearing, grooming, immobility and sedation times were recorded. The number of crossing has been counted.

Statistical analysis

Data were expressed as mean \pm S.E.M. per group. Statistical differences between control and treated groups were tested by two-way repeated measures analysis of variance (ANOVA), followed by Tukey's *post hoc* test. The differences were considered significant at $P < 0.05$. The statistical package used for the analysis was XL Stat 2009.

Results

Preliminary phytochemical test

Our phytochemical studies indicate that decoction of the roots of *Nauclea latifolia* contain flavonoids, phenols, anthraquinones, tannins, glycosides, cardiac glycosids, alkaloids and saponins. Bufadienolids and lipids were found absent (Ngo Bum et al., 2009).

Acute toxicity studies

There were no deaths or any signs of toxicity observed after oral administration of single doses of the decoction of the roots of *Nauclea latifolia* at any dose level up to the highest dose tested (14 000 mg/kg). *Nauclea latifolia* decoction did not produce significant change in behaviour, breathing, cutaneous effects, sensory nervous system responses and gastrointestinal effects in male and female mice. These results showed that, at single dose, there are no adverse effects of *Nauclea latifolia* decoction. These data indicate that the medium lethal dose (LD_{50}) should be higher than 14 000 mg/kg for male and female mice. In contrast, the mortality rate, as well as the acute toxicity, of the intraperitoneally administrated *Nauclea latifolia* decoction increased progressively with increasing doses. The mortality rate of 0% at a dose of 100 mg/kg gradually rose to 100% at 8000 mg/kg, the highest dose studied. The no-observed-adverse-effect level for the intraperitoneal dose was 500 mg/kg, while the lowest-observed-effects level was 750 mg/kg (Alexeeff et al., 2002). Some adverse effects, such as hypoactivity and salivation, were seen immediately after intraperitoneal injection, while others (e.g. anorexia and weight loss) were observed later, and were more pronounced at the highest doses and persisted until death (Table 2). The acute intraperitoneal toxicity (LD_{50}) of the decoction of the roots of *Nauclea latifolia* in mice was 2197.85 mg/kg.

Antipyretic activity

Body temperature

Administration of *Nauclea latifolia* decoction produces an alteration of body temperature in mice. In control group, no significant variations of rectal temperature were detected. Pretreatment with *Nauclea latifolia* decoction at the doses of 80 and 160 mg/kg produced a significant fall of body temperature at 1 h [$F(4,23) = 19.76, P < 0.01$], 2 h [$F(4,23) = 62.8, P < 0.01$], and 3 h [$F(4,23) = 14.2, P < 0.01$], after treatment of animals. At 1 h and 2 h time intervals, the hypothermic effect was significant only at doses of 40, 80 and 160 mg/kg. At 3 h, hypothermia was observed with all doses of *Nauclea latifolia* decoction. The body temperature returned toward basal values after 24 h (Table 3).

Antipyretic test

The data revealed that 160 mg/kg of *Nauclea latifolia* decoction caused a significant reduction [$F(5,28) = 21.71, P < 0.05$] of body temperature up to 1 h after administration. However, the effect increases very significantly for 40, 80 and 160 mg/kg *Nauclea latifolia* decoction until the 3rd h after administration. Acetylsalicylic acid and 160 mg/kg of the extract reduced the fever after 1 h by 0.8 and 0.5°C respectively. Whereas the plant showed effective antipyretic activity 2 h post dosing at 16, 40 and 80 mg/kg, all doses of the decoction of the roots of *Nauclea latifolia* effectively reduced the fever within 6 h. The antipyretic effect was compared with that of standard acetylsalicylic acid (Table 4).

Nociceptive activity

Acetic acid induced abdominal constriction

Table 5 present the pain behavior of writhing response, which was presented as cumulative abdominal stretching response. The protective effect of *Nauclea latifolia* decoction was dose-dependent with 27.3% [$F(7,38) = 124.5, P < 0.05$] reduction observed for 16 mg/kg and 40.8% [$F(7,38) = 124.5, P < 0.05$] seen for 40 mg/kg. The protection offered by 160 mg/kg, 61.7% [$F(7,38) = 124.5, P < 0.001$] was comparable to that of morphine (a centrally acting analgesic), 66.2% [$F(7,38) = 124.5, P < 0.001$]. Acetylsalicylic acid had only 54.3% [$F(7,38) = 124.5, P < 0.01$] inhibition. Naloxone (1 mg/kg, i.p., a non-selective opioid receptor antagonist) significantly reversed (from 61.7% to 19.6%) the antinociceptive effect of *Nauclea latifolia* decoction. Pretreatment with naloxone did not block effectively the protective actions of the extract.

Formalin-induced nociception

Formalin administration produced a typical pattern of flinching and licking behavior. Treating the mice with *Nauclea latifolia* decoction produced a marked and dose-related inhibition of formalin-induced biphasic pain responses in mice. The analgesic effect of this extract at the dose of 160 mg/kg occurred predominantly during the early [$F(6,33) = 227.2, P < 0.01$] and late [$F(6,33) = 239, P < 0.001$] phases. The positive control drug, morphine (5 mg/kg), significantly attenuated both the neurogenic [$F(6,33) = 227.2, P < 0.001$] and the inflammatory [$F(6,33) = 239, P < 0.001$] pain phase, whereas indomethacin (10 mg/kg) was efficient in late phase (67.1% inhibition). Pre-treatment of mice with naloxone (2 mg/kg, i.p.), a non selective opioid receptor antagonist, completely and significantly reversed the antinociceptive effect of *Nauclea latifolia* decoction (160 mg/kg) in both phases of formalin test. Systemic pre-treatment of mice with L-NAME, a NO synthase inhibitor (10 mg/kg, i.p.), also prevented the antinociception produced by the oral administration of *Nauclea latifolia* decoction (160 mg/kg) in the formaline test. Pre-treatment of mice with an ATP-sensitive K⁺ channel inhibitor, glibenclamide (8 mg/kg, p.o.), significantly prevented the antinociceptive effect induced by the oral administration of *Nauclea latifolia* decoction (160 mg/kg) in both phases of formalin test. The adenosine antagonist theophylline (5 mg/kg) however did not have any significant effect of the antinociceptive effect of *Nauclea latifolia* decoction in both phases of formalin test (Table 6).

Tail immersion

As shown in Table 7, all doses of *Nauclea latifolia* decoction, 30 and 60 min after administration cause an increased in tail withdrawal latency. There was a significant reduction of pain sensation induced by tail immersion in warm water. *Nauclea latifolia* decoction at 160 mg/kg produced a significant increase in the withdrawal latencies of the tail as depicted in the time-course curve [$F(7,38) = 52.7, P < 0.001$]. The effect was dose-dependent. At 60 min, *Nauclea latifolia* increased the withdrawal latency by 8.0 ± 0.3 min, (83.3%). Similarly, morphine (5 mg/kg) produced a significant anti-nociceptive effect by increasing the withdrawal latencies of animals [$F(7,38) = 52.7, P < 0.001$] by an average of 8.1 ± 0.5 min, (98.2%). Acetylsalicylic acid had not effect on this test. The anti-nociceptive activities of *Nauclea latifolia* decoction was not effectively blocked by naloxone.

Hot plate

Data indicate that the mean reaction time was highest 1 h after administration of the decoction with all doses used compared to those at 0 and 0.5 h. This reaction was somewhat dose-dependent. Augmentation in reaction time reached 56.1 ± 3.9 min, (230.5%) [$F(7, 38) = 94.21, P < 0.0001$] with 160 mg/kg at 3 h. Pretreatment with naloxone did not effectively reduce the antinociceptive potential of the extract.

Acetyl salicylic acid at 150 mg/kg did not offer any protection against heat-induced pain. Morphine sulphate at 5 mg/kg showed a maximal protective effect of 62.7 ± 1.9 min, (235.3%) [F(7,38) = 117.4, $P < 0.0001$] after 4 h compared to 56.1 ± 3.9 min, (230.5%) [F(7,38) = 117.4, $P < 0.0001$] for 160 mg/kg of the extract after 3 h (Table 8).

Studies of motor coordination (rota-rod test)

In the rota-rod test, *Nauclea latifolia* decoction (16–160 mg/kg) does not exhibit significant effect on mice motor coordination. Only, 320 mg/kg dose of *Nauclea latifolia* at 120 min after treatment, significantly reduced [F(7, 33) = 188.82; $p < 0.001$] locomotors activity (Figure 1).

Antagonism to bicuculline-induced behavioral excitation

Behavioral aspects like locomotion, rearing and grooming were increased by bicuculline. Mice treated with bicuculline presented hyperactivity when compared to controls. *Nauclea latifolia* decoction and diazepam decreased significantly the hyperactivity induced by bicuculline in the open field test (grooming [F(5,28) = 384.5 $P < 0.0001$], rearing [F(5,28) = 40.7, $P < 0.0001$], and immobility [F(5,128) = 93.4 $P < 0.0001$]). The number of crossing was also increased for the same dose (except 16 mg/kg) but only 40, 80 and 160 mg/kg were statistically significant [F(5,28) = 103.57, $P < 0.05$], [F(5,28) = 103.57, $P < 0.01$] and [F(5,28) = 103.57, $P < 0.001$], respectively (Table 9).

Discussion

The hypothermia observed in the present studies after oral administration of *Nauclea latifolia* decoction suggests an implication of both central and peripheral mechanisms. This is not surprising since it is well known that certain psychoactive central nervous system depressant drugs reduce temperature both in normal and pyretic conditions. Studying the antipyretic properties of *Nauclea latifolia* decoction by the yeast-induced hyperthermia test illustrated that the extract was active at sedative and anxiolytic doses (Ngo Bum et al., 2009). These doses (80 and 160 mg/kg) reduced the rectal temperature in hyperthermic mice, one hour after treatment, thus restoring the normal temperature (non-hyperthermic mice). It is currently accepted that prostaglandin E2 (PGE2) is the final fever mediator in brain, especially in preoptic areas of the anterior hypothalamus. It would therefore be interest to determine whether the extract inhibits the syntheses of PGE2 (Li et al., 2008).

Several behavioral nociceptive tests which differ with respect to stimulus quality, intensity and duration, were employed in evaluating the analgesic effect of *Nauclea latifolia* decoction in order to obtain holistic picture of the analgesic properties of the plant extract. The models were selected such that both central and peripheral effects were investigated. At the doses tested, the decoction of the roots of *Nauclea latifolia* was shown to have antinociceptive activity in all the nociceptive models thus indicating that the extract had both central and peripheral-mediated activity. *Nauclea latifolia* decoction produced an inhibition of acetic acid induced writhes. This effect was shown to be significant but not specific (Vogel & Vogel, 1997). Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptor (Stai et al., 1995) or irritation of the visceral surface, which leads to the liberation of histamine, bradykinin, prostaglandins and serotonin (Garcia et al., 2000). It has been reported that the writhing assay is sensitive to Z-opioid and non-steroid anti-inflammatory drugs (NSAIDs) that act primarily by a central and peripheral mechanism, respectively. The NSAIDs can inhibit the number of writhes in this model by inhibiting cyclooxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors by blocking the effect and/or release of anti-inflammatory mediators (Panthong et al., 2007). It is therefore possible that the decoction may act via a mechanism similar to NSAIDs. In the present study, inhibition of acetic acid-induced writhing by the decoction indicates significant antinociceptive activity of *Nauclea latifolia* decoction (Table 4).

It is well known that the formaline test involves two phase: a neurogenic one with the release of substance P and an inflammatory one with the release of serotonin, histamine, bradykinin and prostaglandins (Garcia et al., 2000). The licking response induced by formaline, results from a combination of peripheral input and spinal cord sensitization (Tjiolsen et al., 1992). Taking this into account, the antinociceptive effect of the decoction could be dependent of either peripheral or central sites of action. Central acting drugs, such as opioids inhibit both phases of pain by equally inhibiting the effect produced by prostaglandins released at this level in response to inflammation (Hunskar & Hole, 1987) and by endogenous opioids through their action on the central nervous system. The peripheral analgesics such as acetylsalicylic acid, only inhibit the second phase whereas the narcotic analgesics inhibit both phases. The fact that *Nauclea latifolia* decoction significantly inhibited both phases of the pain induced by the formalin suggests that it may act as narcotic analgesia.

In the present study, the possible mechanism of action of *Nauclea latifolia* roots decoction was investigated in the presence of naloxone, theophylline, L-NAME or glibenclamide. Glibenclamide, an ATP-sensitive K^+ channel blocker blocked the analgesic activities of *Nauclea latifolia* decoction. It is well established that glibenclamide specially blocks ATP-sensitive K^+ channels, with no effect on Ca^{2+} - or voltage-dependent K^+ channels (Amoroso et al., 1990; Edwards & Weston, 1993). Therefore, the present data suggest that the opening of ATP-sensitive K^+ channels plays a role in the analgesic action of *Nauclea latifolia* decoction. The antinociceptive effect of the decoction was also blocked by the nitric oxide synthase inhibitor L-NAME, suggesting that its antinociceptive action involves the

activation of nitric oxide-cyclic GMP pathway at peripheral and/or central levels (Nozaki-Taguchi & Yamamoto, 1998). It has been clearly established that nitric oxide is a downstream signalling molecule released in response to central analgesic such as morphine (Cadet et al., 2004). Hence, the release of nitric oxide or its production is an important step of the antinociceptive action of *Nauclea latifolia* decoction. Naloxone, a non-selective opioid antagonist, reversed the antinociceptive of the decoction in both phases of the formaline test. This finding clearly suggests that activation of opioid receptors and/or an increment of endogenous opioids, either centrally or peripherally, might be involved in the antinociceptive effect of *Nauclea latifolia* decoction (Bjorkman et al., 1990).

To corroborate that *Nauclea latifolia* decoction has analgesic activity, hot plate and tail immersion tests were conducted. In the tail immersion test, which consists of a thermal stimulus, an increase in the reaction time is generally considered to be an important parameter for evaluating central antinociceptive activity (Knoll, 1967). The analgesic activity of *Nauclea latifolia* decoction was observed at a dose of 160 mg/kg and the effect was similar to that of morphine. The tail-flick response is believed to be a spinal mediated reflex (Chapman et al., 1985). The antinociceptive activity of *Nauclea latifolia* decoction in the hot plate test, indicates a central action and likely involves supraspinal as well as spinal components (Yaksh & Rudy, 1976 ; Woolf et al., 1980). Our results show that the antinociceptive effects of the decoction did not vanish but are reduced only slightly after pre-treatment with naloxone. The lack of reduction of the analgesic effects after co-treatment of animals with naloxone suggests that *Nauclea latifolia* decoction acts on the peripheral opioid system and others central receptors involve in pain.

The decoction (16–160 mg/kg) did not attenuate motor coordination (rota-rod performance) suggesting that actions may not be achieved via neuromuscular blockade. Rather, the effects might involve neurons that control central depressant activities. These results suggest that inhibition of pain is not related to the reduction of spontaneous locomotor activity of animals. At the doses (320 mg/kg) which affected locomotion, the decoction also caused an impairment of rod performance, which is indicative of either CNS depressant or muscle relaxant effects (Amos et al., 2005).

The results also show that *Nauclea latifolia* decoction inhibits bicuculline-induced hyperactivity. This is shown by the marked reduction in locomotor activity, the duration of rearing and grooming in the bicuculline-induced behavioral excitation test. This model has been used in laboratory animals to evaluate the central depressant properties of the drugs. The model measures the level of excitability of the central nervous system (Goth, 1984). Agents that suppress this behavior where known to do so through central inhibition. The test is a measure of an exploratory behavior that reveals the sedative activity of the agent (File & Pellow, 1985). Bicuculline, a selective GABA_A antagonist (Sperber et al., 1989) acts directly on the postsynaptic GABA_A receptor complex to induce hyperactivity behavior. The effect of *Nauclea latifolia* decoction against bicuculline suggests a possible interference with central GABAergic neurotransmission. GABAergic inhibitory interneurons are densely distributed in the superficial dorsal horn and at the basis of the longstanding gate control theory of pain, which postulates that loss of function of these inhibitory interneurons (disinhibition) would result in increased pain (Melzack & Wall, 1965).

Preliminary phytochemical screening of *Nauclea latifolia* roots decoction indicated the presence of alkaloids, flavonoids, tannins, saponins and others phytochemical constituents (Table 1). Flavonoids are well known for their ability to inhibit pain perception (Sawadogo et al., 2006). Flavonoids and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever and pain (Baumann et al., 1980). Saponins have been implicated in opioid receptor mechanism (Huong et al., 1995) through antagonistic activity (Wagner et al., 1983) by binding on the sensory nerve terminals. It suggested that flavonoids and saponins were responsible for the antipyretic and antinociceptive effects of *Nauclea latifolia* roots decoction. But it is noted that active components of *Nauclea latifolia* remain to be isolate in the further studies.

The present studies, therefore supports the claims of traditional medicine that *Nauclea latifolia* roots decoction possesses antipyretic, analgesic and anticonvulsant remedy. Overall, these results demonstrate that the central and peripheral effects of *Nauclea latifolia* roots decoction might partially or wholly be due to the stimulation of peripheric opioid receptors through the action of the nitric oxide-cyclic GMP-ATP-sensitive K⁺ (NO/cGMP/ATP)-channel pathway and/or facilitation of the GABAergic transmission.

Footnotes:

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Figure 1

Effects of acute *Nauclea latifolia* decoction (16, 40, 80, 160 and 320 mg/kg) or diazepam (1 mg/kg) treatment on motor co-ordination of mice on the rota-rod, Acquisition process of the rota-rod performance as expressed by means \pm S.E.M. of performance time, ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, significantly different compared to the control, data were analysis by two-way Anova, followed Tukey's (HSD) multicomparison test, n = 6 animals per group.

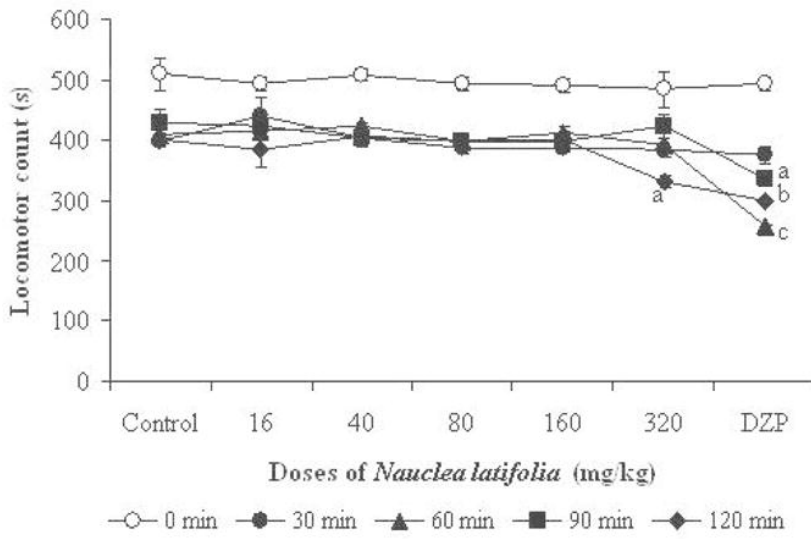


Table 1

Pytochemical constituents of the decoction of the roots of *Nauclea latifolia* .

Constituents/Test	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Phenols	+
Anthraquinones	+
Cardiac glycosids	+
Glycosides	+
Bufadienolids	-
Lipids	-

Key: + = Present; - = Absent.

Table 2

Acute toxicity of the decoction of the roots of *Nauclea latifolia* administered by intraperitoneal injection to mice.

Treatments	Dose (mg/kg)	Sex	D/T	Mortality latency (h)	Toxic symptoms
Control	-	Male	0/5	-	None
		Femelle	0/5	-	None
<i>N. latifolia</i>	100	Male	0/5	-	None
		Femelle	0/5	-	None
<i>N. latifolia</i>	250	Male	0/5	-	None
		Femelle	0/5	-	None
<i>N. latifolia</i>	500	Male	0/5	-	None
		Femelle	0/5	-	None
<i>N. latifolia</i>	1000	Male	0/5	-	None
		Femelle	0/5	-	Hypoactivity, piloerection
<i>N. latifolia</i>	2000	Male	1/5	>36, <48	Hypoactivity, piloerection, salivation, asthenia
		Femelle	1/5	>36, <48	Hypoactivity, piloerection, salivation, asthenia
<i>N. latifolia</i>	4000	Male	3/5	>24, <36	Hypoactivity, piloerection, salivation, syncope
		Femelle	2/5	>24, <36	Hypoactivity, piloerection, salivation, syncope
<i>N. latifolia</i>	8000	Male	5/5	>24, <36	Asthenia, anorexia, salivation, syncope
		Femelle	5/5	>24, <36	Asthenia, anorexia, salivation, syncope

D/T = dead/treated mice; None = No toxic symptoms during the observation period; mortality latency = time to death (in days) after the intraperitoneal injection. The decoction of the roots of *Nauclea latifolia* was to group of mice. Mice in each group were carefully examined for any signs of toxic (behavioural changes and mortality) for 14 days. Control group received distilled water (10 ml/kg, i.p.).

Table 3Influence of the decoction of the roots of *Nauclea latifolia* on body temperature.

Treatments	Dose (mg/kg)	Duration of study (h)						
		t ₀ b	t ₀ a	0.5	1	2	3	24
Control	-	35.2 ± 0.3	35.4 ± 0.1	35.5 ± 0.2	35.3 ± 0.3	35.2 ± 0.1	35.0 ± 0.5	35.5 ± 0.3
<i>N. latifolia</i>	16	35.1 ± 0.2	35.1 ± 0.4	35.3 ± 0.2	34.5 ± 0.1	34.4 ± 0.1	34.1 ± 0.1*	35.3 ± 0.1
<i>N. latifolia</i>	40	35.1 ± 0.2	35.4 ± 0.3	35.3 ± 0.5	34.4 ± 0.2*	34.3 ± 0.1*	34.2 ± 0.1*	35.2 ± 0.1
<i>N. latifolia</i>	80	35.1 ± 0.3	35.2 ± 0.2	35.2 ± 0.2	34.3 ± 0.1**	34.2 ± 0.1**	34.1 ± 0.1*	35.1 ± 0.3
<i>N. latifolia</i>	160	35.2 ± 0.3	35.1 ± 0.3	35.1 ± 0.4	34.3 ± 0.1**	34.2 ± 0.1**	34 ± 0.1**	35.1 ± 0.1

Results are expressed as mean ± S.E.M., t₀ b = initial body temperature, t₀ a = body temperature after the administration of the different treatment, the changes in temperature were expressed as the difference between the reference point and the value of rectal temperature after the injection of the extract or distilled water, n = 6 each group,

* P<0.05,

** P<0.05, significantly different compared to the control group, by two-way Anova followed Tukey's (HSD) multicomparison test.

Table 4Influence of the decoction of the roots of *Nauclea latifolia* on Brewer's yeast-induced pyrexia.

Treatments	Dose (mg/kg)	Duration of study (h)								
		t ₀ b	t ₀ a	0.5	1	2	3	4	5	6
Control	-	35.0 ± 0.1	37.3 ± 0.1	38.1 ± 0.1	38.2 ± 0.1	38.3 ± 0.2	38.4 ± 0.1	38.4 ± 0.1	38.6 ± 0.1	38.2 ± 0.1
<i>N. latifolia</i>	16	35.1 ± 0.1	37.2 ± 0.1	38.0 ± 0.3	38.0 ± 0.3	37.9 ± 0.3*	37.5 ± 0.2*	37.2 ± 0.2*	37.1 ± 0.3*	36.8 ± 0.5**
<i>N. latifolia</i>	40	35.0 ± 0.2	37.2 ± 0.1	38.1 ± 0.1	38.0 ± 0.2	37.4 ± 0.2*	37.0 ± 0.1*	36.6 ± 0.2**	36.1 ± 0.1**	36.0 ± 0.2**
<i>N. latifolia</i>	80	35.1 ± 0.1	37.1 ± 0.1	38.1 ± 0.1	37.8 ± 0.1	37.2 ± 0.1*	36.8 ± 0.1**	36.0 ± 0.2***	35.9 ± 0.2***	35.6 ± 0.3***
<i>N. latifolia</i>	160	35.0 ± 0.1	37.1 ± 0.1	38.0 ± 0.1	37.7 ± 0.2*	37.2 ± 0.1*	36.7 ± 0.2**	35.3 ± 0.1***	35.4 ± 0.3***	35.2 ± 0.3***
Aspirin	300	35.1 ± 0.1	37.1 ± 0.1	37.9 ± 0.1	37.4 ± 0.3*	36.3 ± 0.2***	36.1 ± 0.1***	35.6 ± 0.1***	35.4 ± 0.1***	34.9 ± 0.3***

Results are expressed as mean ± S.E.M., t₀ b = initial body temperature prior to injection of Brewer's yeast, t₀ a = body temperature 18 h after injection of brewer's yeast, n = 6 each group,

* P<0.05,

** P<0.01,

*** P<0.001, significantly different compared to the control groups, data were analysis by two-way Anova, followed Tukey's (HSD) multicomparison test.

Table 5Influence of the decoction of the roots of *Nauclea latifolia* on acetic acid-induced writhing.

Treatments	Dose (mg/kg)	Number of contractions	Inhibition (%)
Control		78.0 ± 4.3	-
<i>N. latifolia</i>	16	56.7 ± 1.3	27.3*
<i>N. latifolia</i>	40	46.2 ± 1.2	40.8*
<i>N. latifolia</i>	80	36.8 ± 2.2	52.8**
<i>N. latifolia</i>	160	29.8 ± 4.1	61.7***
Aspirin	150	35.7 ± 2.4	54.3**
Morphine	5	26.3 ± 1.3	66.2***
<i>N. latifolia</i> + naloxone	160 + 1	62.7 ± 4.1	19.6*

Results are expressed as mean ± S.E.M., the statistical analysis was performed on absolute data, the extract at all doses used began manifesting its assuaging effect on the writhing reflex 1 h following the administration, n = 6,

* P<0.05,

** P<0.01,

*** P<0.001, significantly different compared to the control groups, data were analysis by two-way Anova, followed Tukey's (HSD) multicomparaison test.

Table 6Influence of the decoction of the roots of *Nauclea latifolia* on formalin-induced pain.

Treatments	Dose (mg/kg)	Licking time (s)		Inhibition (%)	
		Early phase (0–5 min)	Late phase (15–30 min)	Early phase	Late phase
Control		73.3 ± 1.8	69.3 ± 2.9	-	-
<i>N. latifolia</i>	16	57.3 ± 3.7	55.2 ± 1.9	21.8*	20.4*
<i>N. latifolia</i>	40	48.2 ± 3.2	45.7 ± 1.3	34.3*	34.1*
<i>N. latifolia</i>	80	33.7 ± 2.3	33.3 ± 2.5	54.1**	51.9**
<i>N. latifolia</i>	160	28.8 ± 1.2	24.8 ± 2.1	60.7**	64.2***
Indomethacin	10	70.2 ± 1.5	22.8 ± 2.2	4.3	67.1***
Morphine	5	35.8 ± 2.2	18.7 ± 1.0	64.8***	73.1***
Theophylline + <i>N. latifolia</i> #	5 + 160	28.0 ± 1.6	25.2 ± 1.5	61.8***	63.7***
Glibenclamide + <i>N. latifolia</i> #	8 + 160	71.5 ± 1.8	68.5 ± 1.6	2.5	1.20
Naloxone + <i>N. latifolia</i> #	2 + 160	71.3 ± 4.0	68.2 ± 2.5	2.7	1.7
L-NAME + <i>N. latifolia</i> #	10 + 160	68,2 ± 1.8	68,5 ± 1.8	7.0	1.2

Results are expressed as mean ± S.E.M., the amount of time spent licking and biting the injected paw was indicative of pain and was recorded in 0–5 min (first phase) and 15–30 min (second phase), n = 6,

* P<0.05,

** P<0.01,

*** P<0.001, significantly different compared to the control group, data were analysis by two-way Anova, followed Tukey's (HSD) multicomparaison test. L-NAME: N_ω-L-nitro-arginine methyl ester.
 # control is *Nauclea latifolia* at the dose of 160 mg/kg.

Table 7

Influence of the decoction of the roots of *Nauclea latifolia* on tail flick response in mice after immersion in 55°C water bath.

Treatments	Dose (mg/kg)	Duration of study (min)			
		0	15	30	60
Control	-	4.4 ± 0.6	4.1 ± 0.6 (-8.6)	4.3 ± 0.5 (-2.8)	4.1 ± 0.1 (-7.1)
<i>N. latifolia</i>	16	4.4 ± 0.6	4.9 ± 0.7 (10.3)	5.1 ± 0.8 (14.9)	5.2 ± 0.8 (17.6) *
<i>N. latifolia</i>	40	4.6 ± 0.4	4.9 ± 0.1 (8.9)	6.2 ± 0.7 (36.5)**	6.5 ± 0.5 (43.3)**
<i>N. latifolia</i>	80	4.3 ± 0.6	5.2 ± 0.4 (20.0)**	6.9 ± 0.7 (62.1)***	7.8 ± 0.5 (81.1)***
<i>N. latifolia</i>	160	4.4 ± 0.8	6.3 ± 0.5 (44.6)***	7.7 ± 0.5 (75.4)***	8.0 ± 0.3 (83.3)***
Aspirin	150	5.9 ± 0.3	6.1 ± 0.3 (2.3)	6.1 ± 0.6 (2.5)	6.0 ± 0.3 (1.8)
Morphine	5	4.1 ± 0.6	6.2 ± 0.4 (51.8)***	8.3 ± 0.5 (103.4)***	8.1 ± 0.5 (98.2)***
<i>N. latifolia</i> + Naloxone	160 + 1	4.4 ± 0.3	5.7 ± 0.4 (30.6)**	6.4 ± 0.3 (47.3)**	6.8 ± 0.3(55.9)**

Results are expressed as mean ± S.E.M. and units are in seconds, percentage of protection against thermally induced pain by warm water are in parentheses, n = 6,

* P<0.05,

** P<0.01,

*** P<0.001, significantly different compared to the control group, data were analysis by two-way Anova, followed Tukey's (HSD) multicomparaison test.

Table 8

Influence of the decoction of the roots of *Nauclea latifolia* on hotplate-induced pain in mice.

Treatments	Dose (mg/kg)	Duration of study (h)							
		0	0.5	1	2	3	4	5	6
Control	-	19.5 ± 0.9	19.3 ± 0.9 (-1.0)	19.1 ± 0.6 (0.2)	19.4 ± 0.9 (0.2)	19.3 ± 0.6 (-0.9)	20.1 ± 0.8 (3.1)	21.1 ± 0.9 (7.9)	20.1 ± 1.0 (3.3)
<i>N. latifolia</i>	16	17.1 ± 1.2	17.3 ± 1.1 (2.0)	20.3 ± 1.4 (30.4)**	22.2 ± 0.7 (30.4)**	25.3 ± 1.3 (48.6)**	21.4 ± 1.6 (25.6)**	20.7 ± 1.2	19.6 ± 1.0 (21.8)** (15.3)*
<i>N. latifolia</i>	40	18.2 ± 0.8	18.8 ± 1.1 (3.3)	22.2 ± 1.3 (31.0)**	23.9 ± 1.2 (31.0)**	26.8 ± 0.9 (46.9)**	22.3 ± 1.4 (22.4)**	20.8 ± 0.9 (14.2)**	20.1 ± 0.8 (10.5)*
<i>N. latifolia</i>	80	17.1 ± 1	17.8 ± 0.9 (4.0)	22.9 ± 1.6 (75.6)***	29.7 ± 1.5 (73.6)***	35.4 ± 4.5 (106.7)***	31.6 ± 3.5 (84.8)***	30.1 ± 2.5 (75.7)***	28.3 ± 1.2 (65.6)***
<i>N. latifolia</i>	160	16.9 ± 1.9	17.8 ± 1.6 (5.0)	26.2 ± 1.3 (172.4)***	46.1 ± 3.5 (172.4)***	56.1 ± 3.9 (230.5)***	47.2 ± 1.7 (178.8)***	46.7 ± 2.5 (175.4)**	45.4 ± 4.3 (167.8)***
Aspirin	150	18.9 ± 1.3	18.9 ± 0.9 (-0.3)	19.3 ± 0.8 (2.8)	19.5 ± 2.5 (2.9)	19.2 ± 1 (1.5)	19.5 ± 1.1 (3.2)	19.2 ± 0.6 (1.6)	19.1 ± 1.2 (0.5)
Morphine	5	18.7 ± 1.6	19.6 ± 1.5 (4.7)	31.6 ± 2.8 (190.0)***	54.2 ± 5.9 (189.9)***	60.1 ± 2.2 (220.9)***	62.7 ± 1.9 (235.3)***	60.6 ± 2.3 (223.8)***	57.9 ± 2.9 (208.3)***
<i>N. latifolia</i> + Naloxone	160 + 1	19.7 ± 1.2	20.6 ± 1.2 (4.7)	24.2 ± 0.7 (44.1)**	28.3 ± 1.3 (44.1)**	30.5 ± 1.3 (55.3)***	26.5 ± 1.5 (34.6)**	27.5 ± 1.0 (39.8)**	28.3 ± 1.8 (44.1)**

Results are expressed as mean ± S.E.M. and units are in seconds, percentage of protection against thermally induced pain by hotplate are in parentheses, n=6,

* P<0.05,

** P<0.01,

*** P<0.001, significantly different compared to the control group, data were analysis by two-way Anova, followed Tukey's (HSD) multicomparaison test.

Table 9Influence of the decoction of the roots of *Nauclea latifolia* on bicuculine induced-behavioral excitation in 1 h.

Treatments	Dose (mg/kg)	Behavioral aspects				
		Crossing number	Duration (s) Rearing	Grooming	Immobility	Sedation
Control 1	-	17.7 ± 0.8	992.2 ± 12.2	31.2 ± 1.8	106.5 ± 4.8	61.7 ± 2.9
Control 2	-	81.8 ± 5.1	2676.7 ± 93.3	73.2 ± 6.2	173.7 ± 3.7	11.8 ± 1.9
<i>N. latifolia</i>	16	68.2 ± 4.5* (16.7)	2110.8 ± 25.3* (21.1)	70.7 ± 3.7 (3.4)	147.2 ± 4.8* (15.2)	26.7 ± 5.3* (55.6)
<i>N. latifolia</i>	40	65.3 ± 4.8* (20.2)	2124.2 ± 6.2* (20.6)	56.8 ± 3.2** (22.3)	142.3 ± 2.8* (18.0)	33.8 ± 4.2* (65.0)
<i>N. latifolia</i>	80	39.2 ± 3.2** (52.1)	1243.8 ± 49.5*** (53.5)	52.3 ± 5.3** (28.5)	130.3 ± 3.1* (24.9)	43.8 ± 4.1** (73.0)
<i>N. latifolia</i>	160	28.3 ± 4.7*** (65.4)	1209.5 ± 55.3*** (54.8)	47.8 ± 3.2** (34.6)	130.3 ± 2.3* (24.9)	65.8 ± 5.8*** (82.0)
Diazepam	3	20.3 ± 1.3*** (75.1)	1116.3 ± 66.4*** (58.3)	37.8 ± 1.8*** (48.3)	119.2 ± 2.2** (31.4)	73.2 ± 5.2*** (83.8)

Results are expressed as mean ± S.E.M., changes in behavioral state of mice were evaluate in termes of changes in number of crossing and duration of rearing, grooming, immobility and sedation, n = 6,

* P<0.05,

** P<0.01,

*** P<0.001, significantly different compared to the control group (control 2), data were analysis by two-way Anova, followed Tukey's (HSD) multicomparaison test. Control 1 has not received bicuculine.