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Analgesic and anti-inflammatory effects of *Cyphostemma vogelii* (Hook. f.) Desc. root extract in mice

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***Cyphostemma vogelii* (family: Vitaceae) is a herbaceous plant which grows in Obukpa town in South Eastern Nigeria. *C. vogelii* is said to be generally medicinal though no specific medicinal activity was mentioned in the literature for this plant. The analgesic effect of *C. vogelii* was evaluated using acetic acid-induced writhing and formalin-induced nociception tests. The extract was also screened for anti-inflammatory activity using carrageenan-induced and kaolin-carrageenan-induced paw edema tests. *C. vogelii* extract dose dependently inhibited acetic acid-induced pain in mice. The extract at 200 and 400 mg/kg significantly inhibited inflammatory and neurogenic pain induced by formalin. The effects of 200 mg/kg on formalin induced pain were similar to those of aspirin, while 400 mg/kg produced more inhibitory effects. At 2, 3 and 5 h post carrageenan injection, 200 and 400 mg/kg extract significantly inhibited paw edema. The extract dose dependently suppressed kaolin-carrageenan-induced edema from 3 h post treatment for up to 24 h. The effect of 400 mg/kg on kaolin-carrageenan-induced edema was similar to that of aspirin, while 100 mg/kg showed the least activity. The data obtained from this study showed that the methanol extract of *C. vogelii* exhibited mild analgesic activity as well as anti-inflammatory activity.**

Key words: Analgesic, anti-inflammatory, mice, *Cyphostemma vogelii,* nociception.

INTRODUCTION

Pain is defined as an unpleasant sensory or emotional experience associated with actual or potential tissue damage (IASP, 1979). Pain occurs when nociceptors of afferent neurons are exposed to noxious stimuli such as trauma or surgery (Busch et al., 2006). In addition to noxious stimulation, nociceptors can be sensitized by algogens released during the inflammatory process (Jones and Hamm, 1977; Snow, 1981). These chemical mediators of inflammatory pain include bradykinin, prostaglandins, substance P, histamine and serotonin (Hughes and Lang, 1983; Boothe, 1984; Dray, 1995).

In modern medical practice, non steroidal anti-inflammatory drugs (NSAIDs) are considered the drugs of choice in the treatment of inflammatory pain (Hosking and Welchew, 1984). NSAIDs inhibit cyclo-oxygenase, the enzyme responsible for the conversion of arachidonic acid to prostaglandin (Lees et al., 2004; Choi and Kwang, 2004). Prostaglandins are known to cause hyperaemia,

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modulate inflammation and sensitize pain receptors (Snow, 1981). Thus, by reducing the amount of prostaglandins, NSAIDs reduce inflammation and the amount of pain felt by animals and humans (Hosking and Welchew, 1984).

However, suppression of prostaglandin synthesis may lead to the gastrointestinal bleeding (Sparkes et al., 2010), acute renal failure (Weir, 2002) and delayed wound healing (Haws et al., 1996; Dvivedi et al., 1997). Therefore, plant derived medicines are gaining acceptance as safer alternatives in the management of painful inflammatory conditions (Choi and Kwang, 2004).

Cyphostemma vogelii (family: *Vitaceae*) is a herbaceous climber seen in rain forests and wooded savannah (Verdecourt, 1993; Burkill, 2000). In Obukpa town, an Igbo speaking area of Enugu state, Nigeria, this plant grows widely and is popularly called “Okoho”. The inhabitants of Obukpa use powders from its root in the preparation of a native soup. Although, *C. vogelii* is said to be generally medicinal, literature search did not reveal any specific medicinal use of this plant. However, several medicinal activities were recorded for a closely related species (C*yphostemma adenocaule*) which grows in Ghana, Tanzania, Kenya and Uganda (Katende et al., 1999; Bosch, 2004). In Tanzania, the leaves of *C. adenocaule* are used to prepare medicines used in the treatment of sore throat, cough and pneumonia (Kokwaro, 1993; Bosch, 2004). In Ghana, Gabon and East Africa, a paste made from its root is applied to treat abscess and inflammation (Bosch, 2004).

Despite ethno botanical reports suggesting that plants in the family *Vitaceae* possess anti-inflammatory effects, there is yet no scientific study conducted to ascertain the anti-inflammatory effect of *C. vogelli*, thus the need for this study. In this study, we investigated the analgesic and anti-inflammatory effects of the methanol root extract of *C. vogelii* using experimental models of pain and inflammation.

MATERIALS AND METHODS

Fresh roots of *C. vogelii* were collected from Obukpa in Nsukka Local Government Area of Enugu State, Nigeria in March, 2011. Samples were authenticated by a taxonomist in the International Centre for Ethnomedicine and Drug Development, Nsukka. The voucher specimen was deposited in the herbarium of the centre.

Preparation of extract

The roots were cut into small pieces, air dried and powdered. The plant materials (500 g) were defatted with n-hexane for 48 h at room temperature, followed by filtration. The residue were dried and subsequently macerated in 80% methanol for 48 h followed by filtration. The filtrate was evaporated to dryness under reduced pressure to obtain the extract of *C. vogelii* (yield = 6.32% w/w). At each time of use, the extract was dissolved in 10% Tween 80 and distilled water to obtain the required concentration of the test solution.

Phytochemical screening

Phytochemical screening was performed using *C. vogelii* extract to qualitatively investigate the presence of alkaloids, tannins, flavonoids, saponin and glycosides (Harborne, 1998). *C. vogelii* extract (1000 mg) was dissolved with 10 ml of distilled water to form a 100 mg/ml test solution which was used for the phytochemical assays.

Acute toxicity

Acute toxicity study was carried out as described by Lorke (1983). Mice weighing 18 to 22 g were randomly assigned to five groups (n = 5) and dosed orally with the extract (100, 200, 400, 800 and 1600 mg/kg). These mice were observed for 48 h for symptoms associated with toxicity such as convulsion, ataxia and diarrhea.

Analgesic studies

Acetic acid-induced abdominal writhing test

The acetic acid induced writhing test was performed as described by Koster et al. (1959). Mice in six experimental groups (n = 5) were treated with *C. vogelii* extract (100, 200 and 400 mg/kg, p.o.), aspirin (200 mg/kg, p.o.) and normal saline (1 ml/kg, p.o.) 1 h before 0.6% v/v acetic acid solution (10 ml/kg) was administered intraperitoneally (i.p.). The numbers of abdominal writhings observed in each mouse were counted for 20 min and recorded (Couto et al., 2011).

Formalin induced nociception test

The test was carried using the method described by Dubuisson and Dennis (1977). Mice were treated with *C. vogelii* extract (100, 200, 400 mg/kg, p.o.), aspirin (200 mg/kg, p.o) and normal saline (1 ml/kg, p.o.). Pain was induced 1 h post treatment by intra plantar injection of 0.05 ml 2.5% v/v formaldehyde solution (Couto et al., 2011). The time spent in licking the paws were recorded in the early phase (0 to 5 min) and late phase (15 to 30 min) after formalin injection.

Anti-inflammatory studies

Carrageenan induced paw edema test

Acute inflammation was induced in the paw of mice by sub plantar injection of 0.1 ml carrageenan (1%), 1 h after administration of *C. vogelii* extract (100, 200 and 400 mg/kg, p.o.), aspirin (200 mg/kg) and normal saline (1 ml/kg, p.o.) as described by Winter et al. (1962). The paw thickness of each mouse was measured using a venire caliper before edema induction and at 1, 2, 3 and 5 h post edema induction. Edema and percentage edema inhibition were calculated as described by Zhang et al. (2008).

Kaolin-carrageenan induced paw edema test

Sub acute inflammation was induced by injecting 0.1 ml of a mixture containing kaolin (20%) and carrageenan (1%) into the hind paw of mice (Hajare et al., 2001). By 18 h post edema induction, the extracts (100, 200 and 400 mg/kg, p.o.), aspirin (200 mg/kg, p.o.) and normal saline (1 ml/kg) were administered to mice in the treatment groups. The treatments were repeated at 3 and 7 h after the first dosing. The paw thicknesses were measured before commencing the treatments (0 min) and at 3, 6 and 24 h after the first treatments were administered. Paw edema was calculated.

Table 1. Phytochemical constituents identified in *C. vogelii*.

Phytochemical constituent	Result
Alkaloids	-
Flavonoids	-
Tannins	+
Glycosides	-
Saponins	+

- (Negative); + (positive).

Table 2. Effect of *C. vogelii* on pain induced by acetic acid.

Treatment (ml/kg)	Number of writhing	Percentage inhibition
Normal saline, 1	106.3 ± 3.7^{d}	0
Aspirin, 200	37.3 ± 7.5^{a}	64.4^{a}
Extract, 100	83.5 ± 3.8^{c}	21.4^{b}
Extract, 200	73.2 ± 2.6^{bc}	31.1^{b}
Extract, 400	62.5 ± 5.5^{b}	41.2^{b}

Different superscripts ^{a,b} in a column show significant difference between group means.

Statistical analysis

Data obtained were presented as mean ± S.E.M. Mean number of writhing, licking time and paw edema in control and extract groups were compared using one way analysis of variance (ANOVA) followed by Duncan multiple range test. The differences were considered significant at $p < 0.05$.

RESULTS

Phytochemical screening

Phytochemical screening showed that the extract contained saponins and tannins, while flavonoids, alkaloids and glycosides were absent (Table 1).

Acute toxicity test

No mortality was recorded in all treatment groups. Also, symptoms such as convulsion, ataxia and diarrhea suggestive of toxicity were not observed.

Acetic acid induced pain

The extract significantly reduced the number of painful writhing induced by acetic acid (Table 2). The inhibitory effect was dose dependent with inhibition of 21.4% recorded for 100 mg/kg, 31.1% for 200 mg/kg and 41.2 % for 400 mg/kg (Table 2).

Formalin induced pain

The extract at 200 and 400 mg/kg significantly inhibited inflammatory and neurogenic pain induced by formalin (Table 3). While the effects of 200 mg/kg on formalin induced pain were similar to those of aspirin, 400 mg/kg produced more inhibitory effects.

Carrageenan induced edema

The paw edema in the treatment groups are shown in Table 4. As shown in this table, treatment of mice with 400 mg/kg extract significantly inhibited paw edema by 1, 2, 3 and 5 h post carrageenan injection. At 2, 3 and 5 h, 200 mg/kg significantly inhibited paw edema while 100 mg/kg inhibited paw edema at 3 and 5 h.

Kaolin-carrageenan-induced edema

As shown in Table 5, the extract dose dependently suppressed kaolin-carrageenan-induced edema from 3 h post treatment for up to 24 h. The effect of 400 mg/kg on kaolin-carrageenan induced edema was similar to that of aspirin, while 100 mg/kg showed the least activity.

DISCUSSION

This study investigated the analgesic effect of methanol root extract of *C. vogelii* using acetic acid-induced writhing test and formalin-induced nociception test. The acetic acid test is often used to assess the peripheral analgesic effect of medicinal plants (Couto et al., 2011). When injected peritoneally, acetic acid stimulates local peritoneal receptors with subsequent release of PGE_2 and $PGF_{2\alpha}$ (Bentley et al., 1983; Deraedt et al, 1980). This test is however a non specific model for detecting peripheral analgesic activity because centrally acting analgesics also positively inhibits pain induced by acetic acid (Trongsakul et al., 2003). Thus, to further investigate the analgesic effect of *C. vogelii,* formalin-induced nociception test, a more satisfactory test for clinical pain was performed (Abbott et al., 1981). The formalin-induced nociception test is used as a model for tonic (Coderre et al., 1990) and inflammatory pain (Tjolsen et al., 1992; Hong and Abbott, 1994). Formalin injection provokes two phases of responses in animals (Lee and Jeong, 2002). Pain in the early phase occurs due to direct stimulation of nociceptors while in the latter phase, pain is due to inflammation (Shibata et al., 1989). Centrally acting analgesics inhibit both phases while peripherally acting analgesics inhibit the second phase (Abram and Olson, 1994; Rosland et al., 1990; Yamamoto et al., 2002). Thus, the ability of 200 and 400 mg/kg *C. vogelii* extract to inhibit acetic acid induced pain as well as both phases of formalin induced nociception

Table 3. Effect of *C. vogelii* on formalin induced pain in mice.

Treatment (ml/kg)	0 - 5 min		15 - 30 min	
	Licking time	Inhibition (%)	Licking time	Inhibition (%)
Normal saline, 1	80.3±7.1^{c}	0	169.3±8.4^{c}	0
Aspirin, 200	54.8±1.9^{b}	31.8^{ab}	89.7±0.6^{ab}	47.0^{ab}
Extract, 100	61.5±7.4^{b}	23.4^{ab}	127.8±9.3^{bc}	24.5^{a}
Extract, 200	45.5±4.8^{ab}	43.3^{ab}	89.8±9.0^{ab}	46.9^{ab}
Extract, 400	36.0±4.9^{a}	55.1^{b}	47.8±9.8^{a}	71.8^{b}

Different superscripts ^{a,b,c} in a column show significant difference between group means.

Table 4. Effect of *C. vogelii* on carrageenan induced paw edema.

Treatment	Paw edema (mm)			
	1 h	2 h	3 h	5 h
Normal saline 1 ml/kg	0.14±0.01^{b}	0.15±0.03^{c}	0.16±0.02^{c}	0.18±0.01^{c}
Aspirin 200 mg/kg	0.11±0.03^{b}	0.07±0.03^{a}	0.06±0.01^{a}	0.05±0.01^{a}
Extract 100 mg/kg	0.13±0.01^{b}	0.12±0.03^{b}	0.12±0.03^{b}	0.10±0.01^{b}
Extract 200 mg/kg	0.13±0.03^{b}	0.11±0.03^{b}	0.11±0.01^{b}	0.09±0.01^{b}
Extract 400 mg/kg	0.06±0.03^{a}	0.08±0.03^{a}	0.06±0.01^{a}	0.06±0.01^{a}

Different superscripts ^{a,b,c} in a column show significant difference between group means.

Table 5. Effect of *C. vogelii* on kaolin-carrageenan-induced paw edema.

Treatment	Dose (mg/kg)	Edema before treatment	Edema post treatment		
			3 h	6 h	24 h
Control	-	0.19±0.01	0.22±0.02^{d}	0.17±0.03^{b}	0.12±0.05^{c}
Aspirin	200	0.17±0.03	0.08±0.02^{a}	0.06±0.02^{a}	0.01±0.01^{a}
Extract	100	0.18±0.03	0.15±0.01^{c}	0.12±0.02^{c}	0.07±0.03^{ab}
Extract	200	0.18±0.02	0.13±0.01^{bc}	0.11±0.02^{c}	0.06±0.01^{ab}
Extract	400	0.18±0.01	0.10±0.01^{ab}	0.08±0.01^{a}	0.04±0.01^{a}

Different superscripts^{a,b,c} in a column show significant difference between group means.

suggests that it exhibited both peripheral and central analgesic effect (Couto et al., 2011).

Carrageenan-induced and kaolin-carrageenan induced paw edema tests were performed to evaluate the effect of *C. vogelii* extract on acute and subacute inflammatory processes (Muruganandan et al., 2001). The injection of carrageenan induces three phases of chemical mediator release which occur in an orderly sequence (Di Rosa, 1972). The initial phase which takes place within 1 to 2 h is mediated by serotonin and histamine, while the intermediate phase is mediated by bradykinin (Zhang et al., 2008). The final phase which occur 2.5 to 6 h post carrageenan injection is presumed to be mediated by PGs (Zhang et al., 2008). Injection of kaolin-carrageenan on the other hand produces inflammation which lasts up to 24 h (Hajare et al., 2001). Edema post kaolin-carrageenan injection is mediated by kinins and activation of kallikrein (Northover and Subramanian, 1961; Bonta and DeVos, 1965). The ability of *C. vogelii* extract to dose dependently inhibit paw edema post carrageenan and kaolin-carrageenan injections shows that it was able to suppress the release of chemical mediators of acute and subacute inflammation (Murunganandam et al., 2001; Hajare et al., 2001; Sini et al., 2010; Udegbunam et al., 2012). Earlier, it has been documented that *Cyphostemma* species contained compounds with anti-inflammatory activity (Bosch, 2004).

Phytochemical screening of the extract of *C. vogelii* revealed the presence of tannins and saponins. Previously, tannins and saponins isolated from medicinal plants exhibited analgesic and anti-inflammatory activities (Thomas et al., 1985; Owoyele et al., 2010; Choi et al., 2005). We thus suggested that the tannins and saponins present in *C. vogelii* extract were responsible for its analgesic and anti-inflammatory activities.

In conclusion, data obtained from this study showed

that the methanol extract of *C. vogelii* exhibited mild analgesic activity as well as anti-inflammatory activity.

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