

Full Length Research Paper

Analgesic, anti-inflammatory and oxytocic activities of *Dracaena steudneri* Engl. stem bark aqueous extract in Wistar rats

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***Dracaena steudneri* Engl. (Dracaenaceae) is a medicinal plant that is used in labour by traditional birth attendants to induce labour, relieve pain and treat various diseases; albeit no documentation. This study determined the analgesic, anti-inflammatory and oxytocic effects of the *D. steudneri* stem bark aqueous extract. *D. steudneri* stem bark was collected, authenticated, oven-dried at 45°C for 48 h, pulverized and extracted using the decoction method. The analgesic effect of the aqueous extract was determined using the acetic acid writhing test, anti-inflammatory activity was determined using paw oedema method while oxytocic activity was determined using abortion and other standard test procedures and were tested at 12.1 24.1 and 48.2 mg/kg. The results of the analgesic study showed that the extract reduced the number of abdominal writhing between 53.51 and 92.40% at 6.02 and 48.2 mg/kg dose, respectively while the standard drug showed a reduction of 36.58% at 10 mg/kg. The anti-inflammatory results showed percentage inhibition of 18.6% (2 h), 22% (2 h) and 18.9% (6 h) at 12.1, 24.1 and 48.2 mg/kg, respectively. The *in vivo* oxytocic activity showed average time taken for the rats to deliver was between 20.63 h at lowest dose (12.1 mg/kg) and 8.25 h at highest dose (48.0 mg/kg) while Oxytocin the reference drug exhibited, 22 h as the average time taken for delivery.**

Key words: Analgesic, oxytocic, anti-inflammatory and obstetrics.

INTRODUCTION

Severe pain during childbirth can lead to physiological adverse effects such as delayed gastric emptying which may increase the risk of aspiration, and/or

hyperventilation (Carlsson et al., 2012). Despite the availability of conventional medicines in the treatment of various diseases and conditions, the use of herbal

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remedies is widely used due to affordability, accessibility and acceptability. Different plants and herbs belonging to various families have been used by traditional birth attendants to induce labour, achieve relatively painless delivery, terminate unwanted pregnancy, and evacuate retained placenta in humans and animals (Akah, 1994). These medicinal plants are usually taken at the end of the gestation period or at the onset of labour (Tenywa et al., 2020).

Dracaena steudneri Engl. is one such plant with a folkloric reputation for use as an oxytocic agent, particularly used when normal delivery is delayed or difficult, and to attain painless delivery during childbirth. *D. steudneri* Engl. belonging to the family Dracaenaceae, is a tree of about 25 m high with a single stem of up to 45 cm in diameter comprising a branched crown. Leaves are clustered at branch ends, leathery, narrowly lanceolate, sessile, 40 to 130 cm long and 4 to 16 cm wide with a narrowed and clasping base bearing attenuated apex towards the acute tip (Fischer et al., 2010). This plant is commonly known as *bush night fighter* locally known as *Sangalyanjovu* among Busoga in Uganda. It is abundant in forest and savannah regions including; Angola, Burundi, Ethiopia, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zambia and Zimbabwe (Burrows and Willis, 2005).

Traditionally, in Tanzania, the leaf of the plant is used to treat hernia, splenomegaly, asthma and chest problems among children, fibroids and infertility in women (Moshi et al., 2007). The decoction of the plant is used to treat malaria (Tabuti et al., 2012) and the treatment of hepatic diseases (Kokwaro, 1993). The decoction of the leaf is also used in the treatment of scars, cough, syphilis, kidney stones and snake bites (Okello and Kang, 2019).

The plant has been reported to possess antifungal activity and inhibited the growth of *Candida albicans*, *Aspergillus spp.* and *Cryptococcus neoformans* from 1.3 to 12 µg/mL. *In vivo* anticandidal activity of the aqueous extract of the plant was reported to have a dose-dependent activity at 100 to 400 mg/kg (Kisangau et al., 2014).

Despite the high rate of evasion as a childbirth remedy, there is no available study on its analgesic, anti-inflammatory and oxytocic activities. Therefore, this study evaluated the analgesic, anti-inflammatory and oxytocic activities of the aqueous extract of *D. steudneri* stem bark in Wistar rats to provide information on its claim during childbirth.

MATERIALS AND METHODS

Collection of plant material

Fresh *D. steudneri* Engl. stem bark was collected from Bususwa village in Jinja district (0.5486472° N, 33.1041479° E)- Uganda, Identified and authenticated by Mr. Protase, a taxonomist at Makerere University, Kampala before the herbarium specimen was deposited at the Makerere University herbarium, Kampala, with

voucher number; 001/MGT.

Preparation of aqueous extract

Stem bark of *D. steudneri* was washed with running water, chopped into small pieces, oven-dried at 45 °C and pulverized mechanically to coarse powder grade (mesh 2000/355). Powdered material was extracted using decoction method in which 250 g of powdered material was weighed into a round-bottomed flask containing 1 L of distilled water and boiled at 80 °C for 45 min. The decocted extract was first filtered with muslin cloth and finally with Whatman filter paper No 1 before it concentrated *in vacuo* using a rotary evaporator (IKA® RV10) at 55°C and later lyophilized using a freeze drier before it was stored at 4 °C until use. A standard concentration of 100 mg/mL was prepared during the experimentation in the animals.

Experimental animals

White Wistar rats of both sexes weighing 120 to 150 g were purchased and housed in the Animal Facility Laboratory of the Mbarara, University of Science and Technology (MUST). The animals were kept under a 12-h light/dark cycle with free access to water and were fed with animal feed pellets purchased from NUVITA, Uganda. They were acclimatized for two weeks prior to the assay. The "Principles of laboratory animal care" (NIH Publication No. 85 to 23, revised 1996) were followed and permission to carry out the research was obtained from the Research Ethics Committee of MUST while the research approval for the study was obtained from the Uganda National Council for Science and Technology with the registration number HS463ES.

Preliminary phytochemical screening

The preliminary phytochemical screening of the *D. steudneri* extract was carried out using standard laboratory procedures, to detect the presence of different secondary metabolites (phytochemical constituents) such as alkaloids, flavonoids, saponins, tannins, glycosides, phenols and terpenoids. These preliminary tests were carried out following the procedures described by Sofowora (1993), Kokate et al. (1995) and Prashant et al. (2011).

Acute toxicity test

The median lethal dose (LD₅₀) of extracts was determined *in vivo* using the procedure described by Lorke (1983). In the first phase, nine rats were randomized into three groups of three rats each and each group received the extract at 10, 100 and 1000 mg/kg. The rats were then observed for signs of adverse effects for 14 days. Since there was no death and observable side effect in the first phase, the second phase was considered. In this phase, four rats were divided into 4 groups of 1 rat each and similarly treated at doses of 1000, 1600, 2900 and 5000 mg/kg orally. The animals were monitored for any sign of toxicity like stretching, rubbing of the nose on the floor and wall of the cage, change in body weight and mortality over a period of 14 days.

Determination of analgesic activity

The analgesic activity of *D. steudneri* extract was evaluated using the writhing test. Inhibition of acetic acid-induced writhing in rats was carried out using the method described by Borgi et al. (2007).

Table 1. Effects of *D. steudneri* stem bark aqueous extract on acetic acid-induced writhing in Wistar rats.

Dose (mg/kg)	No. of writhes	(%) Writhing inhibition
Negative Control (10)	32.75 ± 2.81	-
Aspirin (50)	20.80 ± 2.02	36.58
6.02	15.25 ± 6.24	53.51
12.1	12.80 ± 4.90	61.13
24.1	3.80 ± 2.10	88.41
48.2	2.50 ± 1.20	92.40

Data were expressed as mean ± SD; n=6.

Twenty-four (24) Wistar rats were randomly selected from their cages, divided into 6 groups (I – VI) of 4 rats each and starved overnight to prevent interference of the food with the drug. Group I (positive control) received Acetylsalicylic acid (Sigma Aldrich) at a dose of 50 mg/kg, as a reference drug. The test groups (II – V) received the extract at doses predetermined and extrapolated for human use to clinically relevant doses in the animal model with a conversion factor of 6.2 (Anroop and Shery, 2016) in rats to obtain the given doses of 6.05, 12.1, 24.1 and 48.2 mg/kg body weight respectively. Group VI (negative control) received 10 mL/kg of distilled water. All animals received 0.1 mL of acetic acid (Analar) 15 min post-administration to induce characteristic writhes indicated by the stretching of the abdomen with simultaneous stretches of at least one hind limb. The number of writhes occurring between 5 and 15 min after the injection of acetic acid was recorded for each Wistar rat.

Determination of anti-inflammatory activity

The paw edema technique was used to evaluate the anti-inflammatory activity of the aqueous extract of *D. steudneri* using a method described by Borgi et al. (2007). Thirty (30) male rats were randomly selected from their cages and divided into 6 groups (I – VI) of 5 rats each. Group I (positive control) received 300 mg/kg of acetylsalicylic acid. The test groups were administered with the extract at 12.1, 24.1 and 48.2 mg/kg body weight respectively. Group V (negative control) received 10 mL/kg of distilled water. Fifteen (15) min after administration of the different substances, 0.05 mL of 1% Carrageenan suspension (Borgi et al., 2007) was injected to the left hind paw of all the animals. The paw volume, up to the tibio-tarsal junction, was measured using Vernier calipers (ABS Digimatic Digital Caliper). The measurement was taken at 0h before carrageenan injection and 0.5, 1, 2, 3, 4 and 6h post-injection.

Oxytocic activity test

The *in vivo* oxytocic activity of *D. steudneri* extract was evaluated by a method earlier described by Nworu et al. (2007). Virgin rats (8–11 weeks old) were separated singly and placed with males of proven fertility and breeding potential until a dislodged sperm plug was observed on either the vaginal orifice or on plain white paper placed beneath the cage. Pregnancies were dated from the second day of such observation. The first-trimester group was assigned for the first 8 days of pregnancy; the second-trimester group was assigned for between the eighth and fifteenth day of pregnancy, while the third-trimester group was assigned for between the sixteenth and twenty-first day of pregnancy when the rats were visibly pregnant. The *D. steudneri* extract was orally administered at

12.1, 24.1 and 48.2 mg/kg, control animals received 5 mL/kg normal saline. Oxytocin as the reference drug was given (10 International Units (IU) into 500ml normal saline). The animals were observed for 24 h for vaginal bleeding and fetal and/or placental expulsion. Animals that did not show signs of abortion after 24 h were kept and observed for the length of gestation. Animals were sacrificed and dissected while gross observation of the fetuses and uterus was used to ascertain the possibility of intrauterine death.

Data analysis

All quantitative data were managed using Graphpad Prism5 software and expressed as mean ± standard error of mean (SEM) while the variation in a set of data was analyzed through the one-way Analysis of Variance and the difference among the means was considered at 95% confidence level using the post-hoc method of Newman-Keuls.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of the *D. steudneri* extract showed positive results for alkaloids, tannins, saponins, glycosides, phenols, terpenoids and flavonoids.

Acute toxicity (LD₅₀) test

Wistar rats dosed with aqueous extract of *D. steudneri* during acute toxicity test did not show any sign of mortality or side effects up to 5000 mg/kg within 14 days of observation. Therefore, the LD₅₀ of the aqueous extract of *D. steudneri* in Wistar rats was greater than 5000 mg/kg.

Analgesic activity of *D. steudneri* aqueous extract

Table 1 shows the effects of *D. steudneri* stem bark aqueous extract on acetic acid-induced writhing in Wistar rats.

Table 2. Anti-inflammatory effect of *D. steudneri* in carrageenan-induced paw edema model using a vernier caliper.

Dose of extract(mg/kg)	Change in paw thickness (mm \pm SD, % inhibition)						
	0 h	0.5 h	1 h	2 h	3 h	4 h	6 h
Normal saline (1 mL)	2.64 \pm 0.02	2.99 \pm 0.31	3.47 \pm 0.47	3.87 \pm 0.70	3.90 \pm 0.70	3.95 \pm 0.69	3.97 \pm 0.68
12.1	3.13 \pm 0.05	3.78 \pm 0.26	3.20 \pm 0.16 (7.78)	3.15 \pm 0.25 (18.6)	3.35 \pm 0.27 (14.1)	4.06 \pm 0.12	3.60 \pm 0.10 (9.32)
24.1	3.14 \pm 0.20	3.47 \pm 0.23	3.17 \pm 0.20 (8.65)	3.02 \pm 0.05 (22.0)	3.55 \pm 0.21 (14.1)	3.40 \pm 0.24 (13.9)	3.26 \pm 0.24 (17.9)
48.2	3.12 \pm 0.38	3.54 \pm 0.36	3.35 \pm 0.34 (3.46)	3.38 \pm 0.39 (12.7)	3.33 \pm 0.39 (14.6)	3.25 \pm 0.36 (17.72)	3.22 \pm 0.37 (18.9)
Asprin (300)	2.84 \pm 0.02	3.11 \pm 0.03	2.98 \pm 0.12 (14.1)	3.21 \pm 0.13 (17.1)	3.09 \pm 0.07 (21.8)	3.11 \pm 0.12 (21.3)	3.11 \pm 0.12(21.7)

Data is expressed as Mean \pm SEM; n=6.

Anti-inflammatory activity of *D steudneri* aqueous extract

Table 2 shows the anti-inflammatory effect of *D. steudneri* in carrageenan-induced paw edema model using a vernier caliper.

Oxytocic activity of *D steudneri* aqueous extract

Table 3 shows a dose-dependent activity in the time taken for the Wistar rats to deliver after oral administration of *D. steudneri* stem bark aqueous extract at different dose concentrations. The average time taken for Wistar rats to deliver were 20.63, 15.75 and 8.25 hours at 12.1, 24.1 and 48.2 mg/kg, respectively, while those of negative and positive controls (oxytocin) were 26.75 and 22.0, respectively. Wistar rats that received the extract gave birth earlier /faster than those that received the reference drug. This was dose dependent where by at a dose concentration of 12.1mg/kg (lowest dose), showed an average time of 20.63 h and at a dose of 48.2mg/kg (highest dose) and showed an average time of 8.25 h, while the average time taken for the reference drug was 22.0 h.

DISCUSSION

This study evaluated the analgesic, anti-inflammatory and oxytocic properties of *D. steudneri* stem bark aqueous extract and have shown vigorous activities.

The acetic acid-induced writhing test method used in the evaluation of analgesic activity used in the evaluation of anti-nociceptive activity (Collier et al., 1968; Fujiyoshi et al., 1989) was adopted in this study. It showed that acetic acid produces the constriction reaction of the abdomen which forms a sensitive method for peripheral analgesic agents and this acetic acid promotes an increase in the levels of prostaglandins (PGE2 and PGE2a) in peritoneal fluid, indicating the involvement of the PGEs (Deraedt et al., 1980). However, in this study, aqueous extract of *D. steudneri* stem-bark was able to reverse this effect.

It was reported that analgesics inhibit the cyclooxygenase enzyme (COX 1 and COX 2) which reduces the production of prostaglandins, histamine, Bradykinin and Serotonin (pain mediators) hence leading to the reduction of pain (Sarvesh et al., 2017). This could have happened in the process since *D. steudneri* stem bark in this study exhibited a dose-dependent analgesic activity by significantly reducing the number of

abdominal writhes in the acetic acid pain in Wistar rats after oral administration. The previous study has shown that alkaloids and Flavonoids have anti-nociceptive activity since they possess analgesic activity (Fernanda et al., 2004). Therefore, presence of alkaloids and flavonoids as observed in the extract of *D. steudneri* stem bark during the phytochemical analysis could have contributed to this activity.

The carrageenan-induced acute inflammatory test used to screen for new anti-inflammatory drugs (Babu et al., 2011) which was adopted in this study helped in establishing the anti-inflammatory activity of *D. steudneri* stem bark.

Carrageenan induced paw edema model is also known to be sensitive to cyclooxygenase inhibitors involved in prostaglandins synthesis (Afsar et al., 2015). In the early phase (0-1 h), histamine, serotonin and bradykinin were the first mediators involved whereas prostaglandins are implicated in the second phase (Bhukya et al., 2009; Brooks and Day, 1991). Prostaglandins are responsible for acute inflammation induced by carrageenan as seen in this study. *Dracaena steudneri* might contain some anti-inflammatory agents like flavonoids, phenols, terpenoids and tannins which could be responsible for the blockage of prostaglandins and inflammatory

Table 3. Effects of *D. steudneri* extract on pregnant Wistar rats.

Group	Dose	Average time taken (hours)
Control	10 IU	26.75 ± 0.48
Oxytocin	10 IU	22.00 ± 1.47*
Extract	12.1	20.63 ± 0.24**
Extract	24.1	15.75 ± 0.85****
Extract	48.2	8.25 ± 0.85****

Data were expressed as Mean ±SEM; n=6; * $P<0.05$, ** $P<0.01$, **** $P<0.0001$ compared to negative control.

pathways. In this model of inflammation, *D. steudneri* showed consistent anti-inflammatory activity and thus showed a significant decrease in paw thickness of the Wistar rats.

During labour, the uterus experiences gradual changes in its contractility from one stage to another. The process requires the secretion of both fetal and maternal factors that exert an effect on the myometrium (uterine smooth muscle) responsible for contractions in the uterus. It is reported that induction of labour may be necessary in certain conditions and is advocated in cases of post-term pregnancy, lack of progress, suspected intrauterine growth restriction, pre-eclampsia/eclampsia, pre-labour rupture of membranes at term (Gruber and O'Brien, 2010). In the process of childbirth, oxytocic drugs are seen to be used to induce labour and in the prevention or treatment of post-partum bleeding (Chong et al., 2004). Oxytocin is one of the drugs used for labour induction and it also enhances uterine contractions. This is done by two main modes of action in the uterus. One, oxytocin binds to the myometrium oxytocin receptor to produce contractions and two, it binds to endometrium oxytocin receptor to increase prostaglandin production (Amiera et al., 2014). Prostaglandins are also used to induce labour and to prevent or treat post-partum haemorrhage (Gulmezoglu et al., 2004).

In traditional medicine practice, child delivery always involves the use of potent medicinal herbs to accelerate the process of parturition. Medicinal plants used during childbirth for labour induction have oxytocic activities and are used to induce and maintain labour, help remove retained placenta, regulate post-partum bleeding and used as abortifacients (Tenywa et al., 2020). In this study, *D. steudneri* has been used for the induction of labour in Wistar rats and is seen to exhibit better oxytocic results than the reference drug Oxytocin (Table 3). This confirms its folkloric claims in childbirth by the traditional birth attendants. The results reported oxytocic effects which could be as a result of the phytochemicals present in the plant. *D. steudneri* was seen to contain Tannins and flavonoids which are reported to be contained in the extracts of oxytocic plants and act as antioxidants, antimicrobial that initiate the contractility of the uterus, thus placenta is easily detached and removed from the

uterus (Abdisa, 2018).

Conclusion

This study established the anti-inflammatory, analgesic and oxytocin effects of *D. steudneri* stem bark aqueous extract in the Wistar rats and has validated the folkloric claims of this plant in the treatment of pain, inflammatory and labour induction. However, further study is needed to determine the mechanism of action underlying the analgesic, anti-inflammatory and oxytocic activities. There is need to determine which type of alkaloid is responsible for the oxytocic activity.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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