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EFFECTS OF ETHANOLIC ROOT AND LEAF EXTRACTS OF *CISSAMPELOS MUCRONATA* ON THE EXPECTED ONSET OF PARTURITION IN ALBINO RATS

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ABSTRACT

Further to our earlier in vitro study, the effects of the ethanol leaf and root extracts of *Cissampelos mucronata* A. Rich on the onset of parturition in pregnant albino rats was investigated. Two dose levels of each extract were administered p.o. b.d. to specific animal groups (190-250 g, n=5) from day 19 of gestation until littering. 3 % tween 85 and salbutamol served as controls. The time to onset of parturition was noted for each animal, and any extension in mean gestation length in treated groups relative to that for the negative control group was compared by dose level and extract type relative to the positive control. Both extracts significantly ($p<0.05$) and dose-dependently delayed onset of parturition relative to the negative control, with the root extract eliciting stronger effects. However, only 200 mg/kg b.d. of the root extract showed a significantly ($p<0.05$) greater effect than 0.1 mg/kg b.d. of salbutamol. These results agree with our previous in vitro findings, and directly validate the plant's traditional use as a tocolytic.

KEYWORDS: ***Cissampelos mucronata; parturition; tocolytic***

IINTRODUCTION

Nigeria has been identified by UNICEF as the second largest contributor to the global mortality rate among pregnant women and under-5 year old children [1]. One of the main drivers of high maternal and child mortality rates is preterm labour and birth [2]. While various specific factors contribute to this scenario, the high degree of social and health deprivation associated with the lack of appropriate human, technical and material resources in the most parts of the country [3] is chief. Steer [4] has identified social and environmental factors as more relevant contributing factors to the incidence of preterm birth than race. As such, the rate of preterm births was found to range from 5 % in developed countries to 25 % in developing countries like Nigeria.

Parturition, also called labour and delivery, is the consummation of the reproductive process, and is activated by a combination of endogenous and exogenous factors, the most important of which are hormonal [5, 6]. Where these biochemical interactions occur at term, the chances of maternal and child survival are high; however, the earlier it occurs, the greater the risk of reproductive failure [7]. The same biochemical processes involved in physiological onset of labour are also implicated in preterm labour [8, 9]. As such, many medications that can delay parturition have been successfully used clinically in the management of preterm labour [10, 11], although with associated limitations [12]. Such medications are particularly useful in developing countries like Nigeria, where the incidence of preterm labour is high. Due to the relative scarcity of these agents in rural areas, however, local mid-wives and traditional birth attendants often resort to local herbs to manage threatened abortions and other such obstetrical emergencies [13]. One of the plants used for this purpose in South Eastern Nigeria is *Cissampelos*

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mucronata [14]. In the light of current investigative efforts towards the prevention and treatment of idiopathic preterm labour [15], the study of this plant in this context becomes relevant.

C. mucronata is a climbing shrub that is widespread in dry parts of Africa and the hammocks of Florida and the Everglade keys [16]. The leaves are entire, thickly papery, alternate, and about 8 cm long, while the root is fibrous in nature [17]. A review of its pharmacological profile [18] indicates a plethora of actions including anti-ulcer [19-21], anti-inflammatory [22], spasmolytic [23], antimicrobial [24] and central depressant [25] actions. These actions concern various parts of the plant, especially the leaves and roots. Of greatest interest to the present work is the uterine relaxant property of its root extract [14], an *in vitro* work from which a tocolytic activity was extrapolated in support of its ethnomedicinal use by local birth attendants. This work therefore aims to build on this previous work by seeking to verify *in vivo* the effects of the ethanolic root and leaf extracts of *C. mucronata* on the expected onset of parturition in albino rats as an indication of a tocolytic effect preterm.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh roots and leaves of *C. mucronata* were collected from the wild plant growing in Isuofia, Aguata in Anambra State, Nigeria, by Mr. Goddy Mbonu a traditional medicine healer. Botanical identification of the plant was done by Mr. Anthony Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka.

Extractions

The fresh roots were washed with clean water, cut into smaller pieces, air-dried for seven days and reduced to coarse powder using mortar and pestle. The fresh leaves were also washed and shade-dried for 5 days and reduced into fine powder by pounding in a wooden mortar, after which the stalks and other extraneous matters were removed. About 83 g of the coarse root powder and 120 g of the pulverized leaves was extracted separately for 48 hours by macerating in cold 70 % ethanol with intermittent agitation. Each extract was filtered and the extractive value and percentage yield determined accordingly. Each extract was then screened for phytochemical constituents following standard procedure [26].

Animals and their preparation

Adult male albino mice and mature virgin albino rats (150-180 g) were used for the work. They were obtained from the in-bred stock of the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, and maintained on standard animal diet pellets (Top® feeds) with free access to water. Thirteen mice were used in each case to determine the acute toxicity of the extracts, using the method described by Lorke [27]. The virgin rats were used for the assay of effect on the parturition experiments. The rats were first brought into oestrus by cage-confinement with male albino rats (in separate cages) for 5 days after which they were placed in the same cage with males of proven fertility (2 females per male) for another 4 days to give room for mating. The vaginal plug method as described by Voss [28], and modified by Ochiogu *et al*. [29], was used to confirm successful mating. At the end of the period, the males were removed, and the female rats were monitored for progressing pregnancy, as evidenced by a consistent increase in body weight [30, 31].

Parturition Experiment

Thirty pregnant albino rats (190-250 g) selected from the mated animals were used for the experiment. The animals were randomized into 6 groups (n=5). Groups 1 and 2 served as controls and received 3 % Tween 85 (5 mg/kg) and Salbutamol (0.1 mg/kg) b.d. respectively. Groups 3 and 4 received 100 mg/kg and 200 mg/kg b.d. respectively of the root extract, while groups 5 and 6 received 250 mg/kg and 500 mg/kg b.d. respectively of the leaf extract. Administration commenced from the 19th day of pregnancy, dated from the beginning of mating period, and tocolysis was inferred from the amount of time (in days) that delivery was delayed relative to the negative control, and measured as percentage extension in gestation period.

Statistical analysis

The results were expressed as mean days ± SEM. The mean value for each treatment group was compared to that of the control group for statistically significant difference using the Student's *t*-test at 95 % confidence interval.

RESULTS

Extractive value and percentage yield

The extractive values of both root and leaf extracts were 11.90 g and 5.98 g respectively while their percentage yields were 9.92 % and 7.20 % respectively.

Phytochemical constituents

The phytochemical constituents include alkaloids, steroids, triterpenes, tannins, glycosides, carbohydrates, flavonoids and reducing sugars.

Acute toxicity

The acute toxicity test indicated that the plant extracts are well tolerated, since no death was recorded at the highest dose of 5,000 mg/kg.

Parturition experiment

The results of the parturition experiments are shown in table 1 below. The extracts extended the onset of parturition at all dose levels tested, yielding significant percentage extensions in the mean gestation period observed in the animals that received 3 % Tween 85 (Group 1). However, while all dose levels of both extracts also yield higher percentage extensions in mean gestation period relative to that produced by Salbutamol, only 200 mg/kg of the root extract (Group 3) resulted in a significant extension in this case.

DISCUSSION

Parturition usually occurs in albino rats on the 21st day of gestation [32], as is also confirmed by the results from the negative control group (Group 1). The results obtained from this study showed that both root and leaf extracts of *Cissampelos mucronata* were able to cause dose-dependent delays on the onset of parturition. Since hormonally induced changes in the myometrium and endometrium are involved in preparation of the uterus for implantation, gestation and parturition, the delayed onset of parturition leading to prolonged gestation possibly results from a delayed maturation of hormonal systems and other factors involved in the labour process [33-34]. It is therefore, conceivable that both the ethanolic root and leaf extracts of *C. mucronata* could have achieved these effects by relaxing the myometrium directly, or indirectly by prolonging production and release of progesterone; or even by delaying the steep rise in the oestradiol concentration of blood that subsequently delays the release of posterior pituitary oxytocin.

Myometrial relaxation is partly mediated by stimulation of the β2 receptors through a decrease in myometrial contractility secondary to a fall in intracellular calcium [35, 36]. In our previous work, we found that, in addition to significantly decreasing the amplitude and frequency of non-gravid rat uterine smooth muscles, and completely blocking the spontaneous rhythmic movement of the isolated gravid rat uterus, as any uterine relaxant would be expected to do, the root extract of *C. mucronata* also potentiated the uterine relaxant effect of terbutaline (a typical β2-receptor agonist) [14]. While various mediators, including acetylcholine, serotonin, and prostaglandin E_2, were implicated in these actions, K^+ channel opening events were also found to be involved, implying a non-specific mechanism of action. This agrees with the multifactorial mechanism proposed above.

The possibility also exists of the involvement of factors totally unrelated to the biochemical processes associated with parturition. Garba *et al* [37] have reported the embryofetal effects of the methanolic root extract of *C. mucronata.* These effects were associated with the observed significant increase in resorption sites observed in the uterus of rats that had received 100-300 mg/kg dose levels of the root extract from the 6th to 20th day of gestation. Although the extract was administered for a much longer period than in the present study, the chances of some degree of fetotoxicity still cannot be ruled out. Fetal participation in parturition has been noted for domestic animals, as evidenced by the prolongation of gestation following fetal decapitation [38]. Also multiple births have been associated with a shorter gestation length [39]. Thus, any resorption or any other change that may have occurred in the foetus, as observed by Garba *et al*, could also have contributed to the extension in gestation observed in this study.

The results obtained from this study can be applied to humans because albino rats and humans, although evolutionally very different, have a similar invasive type of placenta [40], as well as many similarities in terms of hormone-responsive gene expression in the uterus [41, 42]. As such, while further work is needed to elucidate the exact mechanisms involved in the observed effects, as well as the specific phytochemical constituent or metabolite involved, since the processes that lead to normal parturition are similar to those associated with

preterm labour, the results of the present study support the ethnomedicinal use of *C. mucronata* as a tocolytic.

CONFLICT OF INTEREST

The authors have no conflict of interest in the conduct and reporting of the research.

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