Effects of ethanolic fruit extract of \textit{Picralima nitida} (Stapf) on fertility of pregnant rats

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Abstract The ethanolic fruit extract of \textit{Picralima nitida} (Stapf) which proved to be a potent spasmogen in vitro was tested in pregnant female Sprague Dawley rats. Fifteen successfully mated female rats were divided into three groups of five animals each. Groups 1 and 2 received intraperitoneal injection of 50 and 500 mg/kg body weight of the extract dissolved in distilled water, respectively, on days 15 and 16 of gestation. Rats in group 3 were given equivalent volumes of distilled water and served as negative control. On the 20th day of gestation, the following parameters: percentage of pregnant females per group (PPF), mean live foetal number (LFN), mean corpus luteum number (CLN), mean resorbed embryo number per pregnant female (REN) and mean day 20 foetal crown-rump lengths (FCRL), were evaluated and fertility index calculated. The extract at 50 mg/kg body weight did not cause any obvious abortion in the treated animals. However, the extract at 50 mg/kg body weight, when compared to the control, significantly ($p<0.05$) reduced the mean FCRL and the fertility index, respectively. On the other hand, the extract at 500 mg/kg body weight caused mortality in all the treated pregnant animals. The histopathology revealed necrosis of the kidney cells. From the study, it was concluded that this extract did not cause any obvious abortion, rather it caused mortality in all the treated pregnant rats.

Keywords \textit{Picralima nitida} · Gestation · Pregnant rats · Toxicity

Introduction

Most of the already-existing abortifacient agents are not cost-effective and have side effects; hence, the search for suitable alternatives is a vital approach in keeping under control the teeming global population (Population bulletin 2004; Pierre et al. 2010). \textit{Picralima nitida} is a medicinal plant with folkloric reputation of influencing uterine muscle function. It has been reported scientifically that the ethanolic fruit extract of \textit{P. nitida} induced myometrial contractions in vitro (Mbegbu 2013). It has also been shown to possess anti-diarrheic (Kouitcheu et al. 2006; Kouitcheu 2007), anti-diabetic (Aguwa et al. 2001), analgesic (Duwiejua et al. 2002), opioid (Menzies et al. 1998), anti-plasmodial (Ezeamuzie et al. 1994), anti-microbial (Fakeye et al. 2004), anti-inflammatory (Obiri 1997; Duwiejua et al. 2002), anti-pyretic (François et al. 1996), trypanocidal (Wosu and Ibe 1989), as well as anti-leishmanial (Iwu et al. 1992) activities. The bark is also used to prepare remedies to male sexual impotence (Adjanohoun et al. 1996). Phytochemical screening of the freshly prepared fruit extract of \textit{P. nitida} revealed the presence of alkaloids, flavonoids, saponins, tannins and glycosides (Obasi et al. 2012). The acute toxicity LD$_{50}$ was estimated at 14.5 and 12.5 g/kg body weight for male and female, respectively. These results showed that prolonged usage of this extract at 1,500–6,000 mg/kg could cause liver, kidney and lung injury, while the effect was mild at small dose levels (750 mg/kg) (Nyunaï and Njifutié 2006). Thus, the extract should be taken with caution bearing in mind that higher doses could affect the tissues (Kouitcheu et al. 2008). \textit{P. nitida} (Stapf) is a bonafide member of the family Apocynaceae and restricted in distribution to African rain forest regions. It is known as \textit{limeme} (Congo), \textit{Eban} or \textit{Obero} (Gabon), \textit{Erin} (Yoruba), \textit{Osugwe} (Igbo) and \textit{Bamborutuk} or \textit{Eban} (Cameroon). Since the in vitro contractile activity of this extract on the uterus has already been established, it is...
worthwhile investigating the in vivo effects of this extract. The objective of the study therefore was to evaluate the abortifacient effect of the aqueous ethanolic fruit extract of P. nitida in pregnant rats on days 15 and 16 of gestation.

Materials and methods

Plant material and its extraction

Mature unripe fruits of P. nitida (Stapf) were collected from Ozubulu, Anambra State of Nigeria by an herbalist. Botanical identification was performed with the aid of freshly collected fruits and leaves, at the International Centre for Ethnomedicine and Drug Development, Nsukka, where a voucher number, INTERCEDD/32, has already been designated for P. nitida (Stapf). Fruits of P. nitida were cut into small pieces, dried and subsequently pulverised with a manual grinder into coarse powder. Three hundred and ninety-five grams of the powder was extracted with 2.5 l of 30 % aqueous ethanol for 72 h, agitated every 2 h. The filtrate was poured into petri dishes, and the solvent was allowed to evaporate at room temperature. The percentage yield was calculated using the following formula: yield=weight of extract divided by the weight of the starting material multiplied by 100. The extract was preserved at 4 °C until usage.

Animals

Fifteen female Sprague Dawley rats, 12–14 weeks of age, weighing between 150 and 200 g, were used to evaluate the abortifacient effect of the extract. The animals were procured from the Laboratory Animal unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Prior to the commencement of the experiments, the animals were acclimatised for a period of 3 weeks. They were kept in metal cages under room temperature, with 12-h light and 12-h dark cycle. Clean water and feed (Vital® growers feed, GCOML, Jos, Nigeria) containing 14.5 % crude protein were supplied ad libitum.

Determination of abortifacient effects of the extract

Each female rat was successively paired with a male rat. A second female rat was introduced into the cage only when mating with the first female rat had been confirmed. Successful mating was confirmed by the presence of a copulatory (vaginal) plug on the floor of the cage in the next morning and/or the presence of whitish flakes (remnants of the copulatory plug) in fresh vaginal smear made on a clean, grease-free microscope slide (Ochiogu et al. 2006). Following mating, the female rat was separated from the male and the day copulatory plug and/or flakes were found designated day 1 of gestation. Thereafter, simple random sampling method was adopted to assign 15 mated female rats into three groups, comprising 5 rats each.

Groups 1 and 2 received intraperitoneal injection of 50 and 500 mg/kg body weight of the extract dissolved in distilled water, respectively, on days 15 and 16 of gestation. Rats in group 3 were given equivalent volumes of distilled water and served as the control.

Determination of fertility index of the extract

On the 20th day of gestation, the following parameters were evaluated according to Wong et al. (1987) and Uchendu et al. (2000):

(a) Percentage of pregnant females per group (PPF)
(b) Mean live foetal number (LFN)
(c) Mean corpus luteum number (CLN)
(d) Mean resorbed embryo number per pregnant female (REN)
(e) Mean day 20 foetal crown-rump lengths (FCRL)

The fertility index (embryo score) was deduced from the equation

\[ \text{FI (fertility index)} = \frac{\text{LFN}}{\text{P}} \times \frac{\text{CLN}}{\text{REN}} \times \text{FCRL} \times \frac{\text{PPF}}{100} \]

Histopathology

Immediate collection of organs required for histopathology was made, and tissue sections of about 3–5 mm thick were fixed for about 12 h in Bouin’s fixative (75 ml saturated aqueous of picric acid, 25 ml of formalin (40 % formaldehyde) and few drops of acetic acid). At the expiration of the 12 h, the tissues were successively dehydrated in ascending grades of alcohol (70, 80, 90 and 100 %) at the interval of 90 min each. Thereafter, the tissues were cleared in xylene twice for 90 min each. After clearing, the tissues were transferred into the infiltrating chambers I and II, containing molten paraffin wax, for 90 min. After this stage, the tissues were embedded in a fresh molten paraffin wax in order to form a hard block. The blocks were then mounted on a microtome and sections of about 5–6 μm thick obtained. The tissue sections were mounted on glass slides coated with 20 % albumin and kept on a dryer at the temperature of 45 °C. The tissues were deparaffinised twice using xylene for 5 min each. Following this, the tissues were rehydrated by rinsing in descending grades of alcohol (100, 100, 95, 95, 80 and 70 %) and water for 10 min. Finally, the tissue sections were stained with haematoxylin and eosin (H&E) and cover slips placed on the dry, ready-to-view slides (Bancroft and Stevens 1977).
Statistical analysis

The computer software Statistical Package for Social Sciences (SPSS) version 15 for Windows was used for the statistical analyses. The data generated were analysed using Student’s $t$ test and the means separated using Duncan’s new multiple range test. The results were presented as the mean with the standard error of the mean (SEM). Differences in the means were considered significant at probability values less than 5% ($p<0.05$).

Results

Percentage yield of the extracts

Percentage yield ($w/w$) was 9.44% of the dry matter. The extract was dark brown in colour and semisolid in consistency.

Abortifacient effects of the extract

An experiment to determine whether the extract could induce abortion in pregnant rats between days 15 and 16 of gestation was conducted. The result showed that while 50 mg/kg body weight of the extract could not produce any noticeable abortifacient effect in the animals, 500 mg/kg body weight of the crude extract caused exudation of fluid from the vulva which soiled the lower abdominal region and death of the treated animals (Fig. 1). Therefore, animals in the group that were treated with 50 mg/kg body weight of the extract still carried their pregnancy to the 20th day of gestation, whereas animals in the group that were treated with 500 mg/kg of the extract died within 24 h of treatment. The gross pathology revealed pale uterine horns with dead foetuses. All the visceral organs appeared normal and there was no rupture of any tissue (Fig. 2). Some vital organs like the liver, spleen, kidneys and lungs were immediately collected for histopathology. The histopathology revealed necrosis of the kidney cells (Fig. 3).

Fertility index of the extract

The result of the fertility index (FI) in this experiment showed that the fertility index of the rats treated with 50 mg/kg body weight was significantly lower than that of the control. The mean foetal crown-rump length (FCRL) of the foetuses harvested from the rats treated with 50 mg/kg body weight was significantly lower than that of the control. However, there was no
statistical difference in the body weight (BW), mean live foetal number (LFN) and mean copora lutea number (CLN) between the rats treated with 50 mg/kg and the control (Table 1).

Discussion

The ethanolic fruit extract of *P. nitida* which proved to be a potent myometrial contractant in vitro did not cause any obvious abortion in pregnant rats dosed with 50 and 500 mg/kg body weight of the extract, respectively, on days 15 and 16 of gestation, rather mortality was recorded in all the rats given 500 mg/kg body weight of the extract. The exudation of fluid from the vulva and the histopathologic lesions showing necrosis of the nephrons/kidney cells suggest that the extract could give rise to acute toxicity of the kidney in pregnant rats in their third trimester. Nyuani and Njiufe (2006) also noticed a similar observation which they termed intrauterine toxicity, but this research further elucidates that this toxicity is particularly of the renal tissues. This is in consonance with Kouitchou et al. (2008) who recorded liver, kidney and lung injury with prolonged administration of this extract at 1,500–6,000 mg/kg body weight. Since this was not a chronic study and the dose was less than that used by the earlier researchers, stress and immunosuppression accompanying pregnancy could have created room for this toxicity and mortality. This could be true because administration of 500 mg/kg body weight of the extract on days 1 to 4 of gestation did not cause mortality (Mbegbu 2013). From the study, it was concluded that even though the extract of *P. nitida* produced myometrial contraction in vitro, the use of this crude extract in vivo is of no physiologic importance due to its tendency of teratogenicity, acute renal toxicity and mortality. It is therefore recommended that further research be carried out in order to isolate and identify the mechanism of toxicity and the toxic principle(s) associated with the crude fruit extract of this plant.

References


Mbegbu EC (2013) Effects of aqueous ethanolic fruit extract of *Picralima nitida* (Stapf) on the uterus of and conception in female Sprague Dawley rats. M.Sc. dissertation, Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. pp 32–38


Table 1  Fertility index of the extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>BW</th>
<th>LFN</th>
<th>CLN</th>
<th>REN</th>
<th>FCRL</th>
<th>FI</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg/kg</td>
<td>262.06±3.87</td>
<td>9.4±0.51</td>
<td>9.6±0.40</td>
<td>0.2±0.2</td>
<td>4.89±0.10</td>
<td>478.8 a</td>
</tr>
<tr>
<td>Control</td>
<td>259.8±3.20</td>
<td>8.6±0.51</td>
<td>8.8±0.37</td>
<td>0.2±0.2</td>
<td>5.31±0.11</td>
<td>518.9 b</td>
</tr>
</tbody>
</table>

Different superscript letters in a column represent significant differences (p<0.05) between groups.

Obiri DD (1997) Studies on anti-inflammatory activity of extracts of seeds of *Picralima nitida*. M. Pharm. degree thesis, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. pp 139


