Oral acute toxicity and estrogenic effects of the extracts of *Passiflora foetida* Linn. (Passifloraceae) leaves in female Wistar albino rats

Bleu Gomé Michel*a, Kouakou Koffi*b, Zahoui Ouga Stanislas*a, Touré Alassane*c, Traoré Flavien*a.

*aLaboratory of Animal Physiology, Biosciences Unit of Formation and Research (UFR Biosciences), University of Cocody, Abidjan, Côte d’Ivoire.

*bLaboratory of Biology of Reproduction and Endocrinology, Biosciences Unit of Formation and Research (UFR Biosciences), University of Cocody, Abidjan, Côte d’Ivoire.

*cDivision of Anatomy-Pathology, Central Laboratory of Veterinary Medicine (LANADA), Bingerville, Côte d’Ivoire.

ABSTRACT

The oral acute toxicity and potential estrogenic activity of the aqueous, hexane and methanol extracts of *Passiflora foetida* leaves were studied in female Wistar rats. After a preliminary phytochemical study, each extract was administered separately at doses of 1000, 2000, 3000 and 5000 mg/kg to 4 groups of 6 adult female rats by oral route to determine the LD₅₀ value. The extracts were then given respectively to 3 groups consisting of 8 adults rats at the dose of 500 mg/kg for 28 consecutive days by gavages. Vaginal smears were examined every morning from each animal to determine the effects of this extracts on the estrous cycle. The estrogenic effect of this plant was carried out by treating orally groups of 6 immature ovariectomized rats with the extracts (500 and 250 mg/kg) and/or 17β estradiol (0.02 mg/kg) for 7 consecutive days. The phytochemical screening showed the presence of sterols, polyterpenes, flavonoids, alkaloids and saponosides in the leaves of *P. foetida*. The oral LD₅₀ values of the three extracts were greater than 5000 mg/kg and no behavioral abnormality was observed in the rats. The extracts also induced a disruption followed by a blockage of the estrous cycle of the rats at the estrous phase. Furthermore, vaginal opening, uterotrophic activity (significant dose dependent increase in uterine wet weight, diameter of uterus, thickness of endometrium, height of endometrial epithelial cells) of the extracts and increase of 17β estradiol-induced uterotrophic effect by this extracts were recorded. In conclusion, the extracts of *P. foetida* leaves had low toxicity by oral route and were found to show estrogenic activity in female Wistar rats.

Key words: *Passiflora foetida*, LD₅₀, estrous cycle, uterotrophic activity, vaginal opening.

INTRODUCTION

*Passiflora foetida* is a medicinal plant which is extensively used in the Ivorian folk medicine. It is one of the 304 high pharmaceutical plants in Côte d’Ivoire where 5000 species have been listed [1]. In this country, its ripe fruits are used as food by the Krobu population in the south [2] and the tribe of Malinke in the North [3]. The leaves and non ripe fruits also serve to treat snake bites, women infertility, epilepsy [2, 4] and abscess [1]. Elsewhere in Africa, the leaves infusions are used to fight hysteria and insomnia in Nigeria [5] and the population of Benin uses aerial parts to cure icteria, hepatitis, constipation, oesophagy and pains [6].
In the Asia Continent, the leaves decoction of this plant is used in India as emmenagogue and to treat asthma, biliousness, hysteria whereas in America, Brazilians use the herbs in the form of lotions or poultices for erysipelas and skin diseases with inflammation [5].

Some pharmacological properties of \textit{P. foetida} have been studied. It was found to have antiparasite, antibacterial, antifungal and antioxidant activities [7, 8, 9, 10]. Furthermore, this plant exhibited hepatoprotective, antidepressant, anticarcinogenic, analgesic and anti-inflammatory properties [11, 12, 13, 14].

The use of \textit{P. foetida} in the treatment of women infertility suggests that this plant could have some estrogenic and/or antiestrogenic properties. Since synthetic estrogens are known to cause endometrial or breast cancer and other adverse effects [15, 16, 17], the use of plants as new natural sources of estrogens is investigated and encouraged.

Thus, the aim of this study was to evaluate the oral acute toxicity and the estrogenic activity of \textit{P. foetida} Linn. (Passifloraceae) leaves in female Wistar albino rats.

**MATERIALS AND METHODS**

**Plant material**
Fresh leaves of \textit{P. foetida} were collected in the North and the South of Abidjan, the economic capital city of Côte d’Ivoire and identified by Dr. N’Guessan K. (Laboratory of Botany, University of Cocody, Abidjan). A voucher specimen is deposited in the botanical garden of this University under the number 746B.

**Preparation of the extracts**
The collected plant material was dried at an ambient temperature (30±2°C) without exposure to sun light and crushed to obtain a powder which was divided into three parts. The different parts (50 g of each) were macerated separately for 24 hours in water (1500 ml), hexane (750 ml) and methanol 95° (750 ml), filtered using Whatman filter paper number 1 and concentrated in an air circulating oven at 50°C until total dryness. The aqueous, hexane and methanol extracts obtained (yield 20.23%, 6.83% and 28.03% respectively) were then stored at 4°C in a refrigerator for the experimental studies.

**Experimental animals**
Female immature (30-40 g) and adult virgin (120-140 g) Wistar albino rats were obtained from the Biosciences Unit of Research and Formation (UFR Biosciences) of the University of Cocody, Abidjan. The rats were maintained in a room at a constant temperature (24±2°C), a photoperiod of 12 hours natural light and 12 hours dark. The hygrometry was 50-55% and they were free allowed to water and food (Ivograin, Abidjan, Côte d’Ivoire).

**Phytochemical study**
The phytochemical screening was done using classic methods [18]. Chemical compounds tested in the three extracts of \textit{P. foetida} leaves were: sterols, polyterpenes, polyphenols, flavonoids, tannins, quinones, alkaloids and saponosides.

**Oral acute toxicity**
The adult female rats were divided into 4 groups of 6 animals for each extract and administrated orally the single doses of 1000, 2000, 3000 and 5000 mg/kg body weight, using an intragastric cannula. The control group was also consisting of 6 rats and received olive oil. The maximum dose volume administered did not exceed 2 ml/100 g body weight. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 consecutive days for behavioral changes and mortality [19].

**Effect of \textit{P. foetida} extracts on the estrous cycle**
The adult female rats with regular estrous cycle (4-5 days) were divided into 4 groups of 8 animals each. The first group served as control and received orally olive oil. The treated groups were given by gavage a dose of 500 mg/kg body weight of the aqueous extract, the hexane extract and the methanol extract respectively. All the treatments were given daily until a period of 28 days. Vaginal smears were examined every morning between 8:00 and 9:00 a.m., from each animal which was weighed every week. Smears were prepared as described by Sahar et al. (2007) [20]. The staining technique of Harris-Shorr was used to stain the smears [21].
Estrogenic activity
The immature female albino rats were bilaterally ovariectomized (ovx) under light ether anesthesia through lateral incisions in the skin just below the last rib [22, 23]. After a post-operative rest period of 7 days, the rats were divided into 11 groups of 6 animals each and treated orally with:
- Group I: control (olive oil)
- Group II: 17β estradiol (0.02 mg/kg body weight)
- Group III: aqueous extract (500 mg/kg body weight)
- Group IV: aqueous extract (250 mg/kg body weight)
- Group V: aqueous extract (500 mg/kg body weight) + 17β estradiol (0.02 mg/kg body weight)
- Group VI: hexane extract (500 mg/kg body weight)
- Group VII: hexane extract (250 mg/kg body weight)
- Group VIII: hexane extract (500 mg/kg body weight) + 17β estradiol (0.02 mg/kg body weight)
- Group IX: methanol extract (500 mg/kg body weight)
- Group X: methanol extract (250 mg/kg body weight)
- Group XI: methanol extract (500 mg/kg body weight) + 17β estradiol (0.02 mg/kg body weight).

All the above treatments were given for 7 days. After 24 hours following the last treatment, they were sacrificed by decapitation under light ether anesthesia. The uteri were dissected out and weighed on a sensitive balance. Estrogenic activity was assessed according to the method of Evans et al. (1941) and Edgren and Calhoun (1957), taking uterine wet weight and vaginal opening [24, 25]. Additionally, the uterus of each rat was fixed in bouin’s fluid for 24 hours. The tissues were dehydrated and embedded in paraffin. The paraffin sections were cut at 4 µm and stained with haematoxylin-eosin for histological examinations. The diameter of the uteri, thickness of endometrium and height of endometrial epithelial cells were measured using an ocular micrometer [26].

Statistical analysis
The data were expressed as mean±SEM. Statistical analysis of the variance between control and experimental values were done using student’s t-test. A probability level of less than 5% (p<0.05) was considered significant.

RESULTS

Phytochemical study
Results of phytochemical screening are presented in the table 1. The content of P. foetida leaves depends to the solvent used for the extraction.

Table 1: phytochemical screening of the extracts of P. foetida leaves

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Tests used</th>
<th>AE</th>
<th>HE</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols-polyterpenes</td>
<td>Liebermann test</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>FeCl₃ test</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Cyanidin test</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>Stiasny test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>Borntraeger test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>D. and B. test</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Saponosides</td>
<td>Foam test</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

AE: aqueous extract; HE: hexane extract; ME: methanol extract
- = absence; + = low presence; ++ = high presence; D=Dragendorff; B=Bouchardat.

Acute toxicity
No lethality or behavioral changes were observed 14 days after the treatment of the rats with the doses from 1000 to 5000 mg/kg body weight. Hence, the dose of 500 mg/kg body weight, ten times less than 5000 mg/kg was used as maximal dose for the rest of the experiments.

Effect on the estrous cycle
The three extracts of P. foetida induced a disruption followed by a blockage of the estrous cycle at the estrous phase in 100% of the rats treated with the aqueous extract and 75% of those treated with the methanol and the hexane extracts. For the 28 days of the treatment, the total duration of the estrous phase was highly significantly increased (p<0.001). Both the metestrous and the diestrous phases were highly significantly decreased (p<0.001); but no
significant variation of the duration of the proestrus phase was recorded when compared with the control group (figure 1).

![Bar chart showing total duration of estrous cycle phases](chart.png)

**Figure 1:** Total duration of the phases of the estrous cycle during the 28 days of the treatment at the dose of 500 mg/kg of the extracts of *P. foetida* leaves.

Values are presented as means±SEM (n=8/group); ***p<0.001. AE = Aqueous extract, HE = Hexane extract, ME = Methanol extract. The extracts of *P. foetida* leaves induced significantly the increase of the total duration of the estrous phase and decreased significantly the duration of metestrous and diestrous phases of the estrous cycle of adult Wistar rats.

**Table 2:** Body weight gains of the female rats treated with the extracts of *P. foetida* leaves at a dose of 500 mg/kg for four weeks.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>108.07±4.95</td>
<td>118.95±7.72</td>
<td>123.50±8.12</td>
<td>129.81±13.05</td>
</tr>
<tr>
<td>AE</td>
<td>104.48±6.55</td>
<td>123.41±6.8</td>
<td>125.84±4.88</td>
<td>127.21±6.17</td>
</tr>
<tr>
<td>HE</td>
<td>101.19±9.46</td>
<td>116.11±8.42</td>
<td>122.43±10.43</td>
<td>125.32±11.54</td>
</tr>
<tr>
<td>ME</td>
<td>105.83±5.59</td>
<td>124.90±9.72</td>
<td>130.24±6.77</td>
<td>132.20±7.65</td>
</tr>
</tbody>
</table>

Values are presented as means±SEM (n=8/group); initial weight gain before treatment was considered as 100% for all the rats. AE = Aqueous extract, HE = Hexane extract, ME = Methanol extract. The extracts of *Passiflora foetida* leaves did not induce any significant changes in the body weight gains of the rats.

**Effect on the estrous cycle**

The three extracts of *P. foetida* induced a disruption followed by a blockage of the estrous cycle at the estrous phase in 100% of the rats treated with the aqueous extract and 75% of those treated with the methanol and the hexane extracts. For the 28 days of the treatment, the total duration of the estrous phase was highly significantly increased (p<0.001). Both the metestrous and the diestrous phases were highly significantly decreased (p<0.001); but no significant variation of the duration of the proestrus phase was recorded when compared with the control group (figure 1).

**Body weight**

The body weight of all the rats increased progressively from the week 1 to the week 4 of the treatment but weight gains of the treated groups were not significantly different from those of the control group (table 2).

**Estrogenic activity**

The estrogenic effects of the extracts of *P. foetida* on immature ovx rats are presented in the table 3. The uterine wet weights of the treated rats were dose dependently significantly increased by the aqueous extract, hexane extract and
methanol extract and by 17β estradiol. Simultaneous administration of the aqueous extract (500 mg/kg) and 17β estradiol (0.02 mg/kg) induced a weak non significant increase of the uterine wet weight compared to 17β estradiol alone treated rats. However rats treated simultaneously with 17β estradiol and methanol extract or hexane extract (500 mg/kg) showed significant increase in uterine wet weight when compared to 17β estradiol treated group. The uteri of the treated groups were inflated and fluid filled, resembling to proestrus/estrous uterus.

Histometric examinations of the treated rats showed significant dose dependent increase of the diameter of the uterus, the thickness of endometrium and the height of endometrium epithelial cells (table 3). The endometrium was proliferated and the lumen dilated. The epithelium of the endometrium consisted of tall columnar cells. Apoptosis and/or mitotic figures were observed within the luminal and glandular epithelium. Vacuolar degenerations and polymorphonuclear cells were also observed in the lamina propria. All the changes in 17β estradiol alone treated group were less than those observed in simultaneous extract and 17β estradiol treated groups. The uteri of the control rats showed atrophic endometrium with small epithelial cells and reduced lumen (figure 2). Vaginal opening was also recorded in treated rats whereas the control rats did not exhibit any opening of vagina.

Table 3: Effects of the extracts of *P. foetida* leaves and 17β estradiol (0.02 mg/kg) on the uterus of immature ovx Wistar rats after 7 days of treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Uterine wet weight (mg/100 g b.w.)</th>
<th>Diameter of uterus (µm)</th>
<th>Thickness of endometrium (µm)</th>
<th>Height of epithelial cells (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (olive oil)</td>
<td>15.78±2.13</td>
<td>434.49±163.26</td>
<td>109.74±20.03</td>
<td>17.26±2.00</td>
</tr>
<tr>
<td>17β estradiol</td>
<td>100.32±25.55**</td>
<td>1251.54±370.40**</td>
<td>329.97±17.58**</td>
<td>36.04±4.86***</td>
</tr>
<tr>
<td>AE (500 mg/kg)</td>
<td>188.69±122.73***</td>
<td>1369.82±122.73***</td>
<td>337.74±115.77**</td>
<td>43.25±7.02***</td>
</tr>
<tr>
<td>AE (250 mg/kg)</td>
<td>30.39±5.44***</td>
<td>685.29±107.43*</td>
<td>194.90±32.11**</td>
<td>21.41±1.47**</td>
</tr>
<tr>
<td>AE (500 mg/kg) + 17β estradiol</td>
<td>105.54±25.11***</td>
<td>1297.65±90.96***</td>
<td>369.15±83.97***</td>
<td>39.08±5.44***</td>
</tr>
<tr>
<td>HE (500 mg/kg)</td>
<td>121.91±27.22***</td>
<td>1460.61±245.97**</td>
<td>279.09±75.82**</td>
<td>36.06±5.11***</td>
</tr>
<tr>
<td>HE (250 mg/kg)</td>
<td>26.91±3.88**</td>
<td>713.43±197.21*</td>
<td>139.02±12.36*</td>
<td>22.32±1.84**</td>
</tr>
<tr>
<td>HE (500 mg/kg) + 17β estradiol</td>
<td>133.16±16.87***</td>
<td>1487.36±201.48***</td>
<td>339.14±101.87**</td>
<td>40.70±5.33***</td>
</tr>
<tr>
<td>ME (500 mg/kg)</td>
<td>36.59±7.76**</td>
<td>705.08±116.61*</td>
<td>129.80±21.00</td>
<td>29.40±3.94***</td>
</tr>
<tr>
<td>ME (250 mg/kg)</td>
<td>23.63±4.01**</td>
<td>638.74±160.31*</td>
<td>122.29±18.23</td>
<td>22.18±1.45**</td>
</tr>
<tr>
<td>ME (500 mg/kg) + 17β estradiol</td>
<td>143.00±37.81***</td>
<td>1348.69±317.19***</td>
<td>352.18±67.62***</td>
<td>48.84±5.12***</td>
</tr>
</tbody>
</table>

Values are presented as mean±SEM (n=6/group); *p<0.05, **p<0.01, ***p<0.001; b.w. = body weight. The extracts of *P. foetida* leaves induced significant dose dependent uterotrophic effects in immature ovx rats.

**DISCUSSION**

The oral acute toxicity study of *P. foetida* leaves indicated that this plant did not induced any mortality or change of behavior in female adult Wistar albino rats for the doses less than 5000 mg/kg. The median lethal dose (LD₅₀) of the three extracts must be above 5000 mg/kg. According to Schorderet (1992) [27], substances with LD₅₀ values greater than 5000 mg/kg are classified as substances with low toxicity. Thus, the aqueous extract, hexane extract and methanol extract of *P. foetida* leaves can be considered as substances with low toxicity.

The estrous cycle of rats consists of four phases (estrus, metestrous, diestrous and proestrus) and the mean duration of one cycle is 4 to 5 days [28]. The ovulation occurs from the beginning of proestrus to the end of estrus [29]. The analysis of vaginal epithelium cells by vaginal smears are used for the determination of the phases of estrous cycle [30, 31]. Three types of cells could be recognized: epithelial cells (round and nucleated), cornified cells (without nucleus) and the little round ones (leukocytes). The proportion among them was used for the determination of the estrous phases. A proestrus smear consisted of predominance of nucleated epithelial cells; an estrus smear primarily consisted of anucleated cornified cells. A metestrus smear consisted of the same proportion among leukocyte, cornified and nucleated cells. A diestrus smear primarily consisted of a predominance of leukocytes [20, 32, 33]. In this study, the three extracts induced significant increase in the estrous phase duration and decrease of metestrous and diestrous phases. These effects are similar to those of 17β estradiol at a dose of 0.02 mg/kg [34]. Estrogen is a
steroid hormone which is secreted by granulosa and theca interna cells in the ovaries [35]. Vaginal epithelium is one of the target tissues of this hormone where it induces cell keratinization [36]. The effects of *P. foetida* extracts on the estrous cycle may be due to the presence in these extracts of some estrogenic substances. Thus, we tested these extracts in immature ovx rats to carry out the estrogenic or antiestrogenic action of this plant. Results showed that 17β estradiol and all the extracts increased significantly the uterus wet weight, the diameter of uterus, thickness of endometrium and height of epithelial cells. These effects of *P. foetida* are similar to those of the phytoestrogen genistein [37], the ethanolic extract of *Cassia occidentalis* [26] and various extracts of *Plumbago zeylanica* roots [38] on immature female Wistar albino rats. Furthermore, the uterotrophic effects of 17β estradiol were amplified by the presence of the extracts. In uterus, estrogen stimulates endometrial proliferation which results in endometrial hyperplasia [39]. Therefore, the extracts could act as estrogen-like and as estrogen agonists, potentiating its action. The same effects have been obtained with *Citrus medica*. The petroleum ether extract of this plant increased significantly the ethinyl estradiol-induced uterotrophic effect in immature ovx albino rats [40]. Additionally, the precocious vaginal opening induced by the extracts confirmed the estrogenic profile of these extracts which mimic estrogen effects. However the non significant difference in body weight gain between control and *P. foetida* treated groups during the experiments indicated that the extracts did not affect the growth of the rats.

![Figure 2: Photomicrograph of the uterine endometrium and lumen of the ovx control and HE treated rats.](image)

Control rats showed atrophic endometrium and reduced lumen (A). Rats treated with 17β estradiol (B) or the hexane extract of *P. foetida* leaves (C) showed proliferated endometrium and dilated lumen. However, the endometrium of the simultaneous 17β estradiol and hexanic extract treated rats (D) were more proliferated than those of 17β estradiol alone treated rats. E=Endometrium, EL=Endometrium lumen. Original magnification X 10, stain: Haematoxylin-eosin.

Preliminary phytochemical screening indicated the presence in *P. foetida* leaves of sterols, polyterpenes, polyphenols, flavonoids and alkaloids which are known for their estrogenic effects on uterus and vagina of rats and mice [37, 41, 42]. The effects of *P. foetida* on vagina and uterus could be explained by the presence in this plant of these compounds and they act probably by the same mechanism of action of genistein which induced expression of estrogen gene receptor C-fos and the complement C3 [43, 44, 45, 46].
CONCLUSION

The extracts of *P. foetida* leaves were had low toxicity by oral route and were found to show estrogenic activity in female Wistar albino rats. This plant merits further investigations for identification of the active constituents and for use as source of hormone replacement therapy.

REFERENCES

[34] K Kouakou. Thèse de Doctorat 3ème cycle, Université de Cocody (Abidjan, Côte d’Ivoire, 2000)