Effects of ethanolic extract of *Rhynchosia sublobata* (Schumach) Meikle on estrous cycle in Wistar rats

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Abstract: The effect of *Rhynchosia sublobata* aqueous leaf extracts on female reproductive cycle was studied in sixteen (N=16) sexually matured female wistar strain rats with regular estrous cycle. Rats were randomly divided into four (4) groups of four (n=4) rats per group. The experimental groups were treated as follows: Group I treated with 5000mg/kg, Group II with 2500mg/kg and Group III with 1000mg/kg of the aqueous *Rhynchosia sublobata* leaf extract and Group IV (control) received no treatment. The estrous cycle changes were determined by daily observation of vaginal smear. There was an increase in the percentage of rats going into prolong proestrus and estrus phases of the estrous cycle with a corresponding decrease in the phases of metestrus and diestrus in all treated groups. The increase in the duration of the two phases of the cycle has a dose dependent response. Treatment with the highest dose 5000mg/kg of the extract resulted in a significant increase in the duration of the proestrus and metestrus phases compared with the control group. Results showed that *R. Sublobata* has the potential for use as an antifertility agent in pet animals in future, which require more studies concerning the pharmacological properties of *R. Sublobata* in more details. The observed claims on *R. Sublobata* by pastoralists have been scientifically validated.

Keywords: *Rhynchosia Sublobata*, reproductive cycle, antifertility agent, vaginal smear.

Introduction

The short estrous cycle length in the female rat makes them an ideal laboratory animal for investigation of the changes occurring during the reproductive cycle (Spornitz et al. 1999; Marcondes et al. 2001). Many plant species consumed by animals are known to affect their estrous cycle, especially the leguminous plants due to their phytoestrogenic components (Edwin et al. 2008; Lindner 1976). These leguminous plant species are on the increase as a result of demographic changes associated with environmental degradation due to uncontrolled farming activities and settlements. These activities restrict the pastoralists to certain limited grazing areas where palatable pasture have become extinct and successively replaced by unpalatable and to some extent poisonous leguminous plant species. Such changes are noticeable in Gujba Local Government Area of Yobe State located on longitude (10° 25' N and 11°N) and latitude (13° 45' E and 14° 18' E), it lies within the sahel savannah zone. The wet season begins from April to September with an annual average rainfall of 794.4mm (31.28”) while dry season spans from October to March annually.

At the beginning of the wet season, the pastoralists complain from the increasing incidence of poisoning cattle due to ingestion of a certain leguminous plant that was later identified as *Rhynchosia sublobata*, the plant grows rapidly and thrive well between the months of May and June. It was observed to cause sporadic abortions in cattle, delivery of physically weak...
calves and cows exhibit long calving intervals. However, there is few published information on the effect of this plant on the reproductive parameters and on oestrous cycle in animals. Therefore, this study was conducted to determine the effect of *Rhynchosia sublobata* on the oestrous cycle of female Wistar rats and to validate the claims by pastoralists in Gujba Local Government Area on the observed effects of the plant in laboratory animal model.

### Materials and methods

#### Experimental animals

Sixteen sexually matured female Wistar rats (*Rattus norvegicus*), weighing between 87-104g obtained from the Department of Veterinary Medicine and Surgery, Ahmadu Bello University, Zaria, Nigeria, were used. The animals were housed in plastic cages with sawdust as bedding, they were fed a standard laboratory diet twice daily and watered *ad libitum*. Rats were exposed to a 12 hours light/dark cycle at a laboratory temperature of 34 ± 2°C. The rats were quarantined for 14 days before the commencement of the study. They were identified by different colour markings on their foreheads. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

#### Collection and identification of plant

Plant material were collected during early wet season from Gujba Local Government Area and identified as *Rhynchosia sublobata* by a plant taxonomist at the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria. Voucher specimen number 866 was deposited at the herbarium of the Department for future reference.

The leaves of *Rhynchosia sublobata* were shade dried at room temperature in the laboratory at the Department of Pharmacognosy, Ahmadu Bello University, Zaria. The dried leaves were powdered using a wooden mortar and pestle then weighed and stored in water proof and airtight container until used.

#### Extraction of plant material

Powdered plant material weighing 500g was then extracted with 95% ethanol and allowed to stand for 48 hours and filtered through a funnel blocked with cotton wool. The resulting dark brown liquid was evaporated to dryness at a temperature of 60-70°C in a water bath (Buchi, Rotavapor R-210, Laborteknik, AG, Flavil, Switzerland). The powder obtained was further extracted with 600ml of hot water then evaporated to dryness, the little moisture was finally dried off in a dessicator (Sofowora 1993).

#### Acute toxicity study

Acute toxicity test were carried out on four mice according to OECD guidelines (OECD 2004). Extracts were prepared in deionised water at different doses up to 5000 mg/ kg bw was administered orally. The experimental animals were observed for any sign of behavioural changes, any toxicity and mortality up to 48 hours.

#### Animal treatment schedule

A total of sixteen (N=16) rats were blocked according to weights and randomly divided into four (4) groups containing four rats each (n=4). An insulin syringe (1ml) fitted to curve 18-gauge needle with blunt end was used for the administration of the extract dissolved in distilled water. The experimental groups were administered different doses as follows:

- Group I received 5000 mg/kg body weight of the extract
- Group II received 2500 mg/kg body weight of the extract
- Group III received 1000 mg/kg body weight of the extract
- Group IV (control) received distil water.

#### Determination of normal estrous cycle

The phases of estrous cycle were determined by daily examination of vaginal smear as described by (Marcondes et al. 2002), with slight modification of staining the slides with methylene blue or Geimsa stains to have clear view of
the cells. The phases of the oestrous cycle of the rats were identified as described (Marcondes et al. 2002) and classifying experimental animals into the different phases of estrous cycle as adopted (Edwin et al. 2008).

**Statistical analysis**

Data collected were subjected to a simple descriptive statistical analysis using graph pad (GraphPad 2000). P values < 0.05 were considered statistically significant where treatments were found to have significant effect on response variable.

**Results**

There were neither signs of toxicity nor mortality, so the extract proved to be safe. Based on LD₅₀ studies, doses of 5000 mg/kg b/w, 2500 mg/kg b/w and 1000 mg/kg b/w were selected for the experimentation.

**Figure 1:** **Plate A:** Photomicrograph of vaginal smear from female rat at proestrus phase of the cycle, showing predominance of nucleated cells (arrowed). **Plate B** Photomicrograph of vaginal smear from female rats at estrus phase of the cycle, showing predominance of epithelial cells (arrowed). **Plate C:** Photomicrograph of vaginal smear from female rats at metestrus phase of the cycle, showing nucleated epithelial (E), Cornified (C) and Leukocyte (L) cells. **Plate D:** Photomicrograph of vaginal smear from female rats at diestrus phase of the cycle, showing predominance of leukocyte cells. × 350. (Methylene blue stained)
Majority of the rats in Group I treated with 5000 mg/kg body weight and Group II treated with 2500 mg/kg body weight of the extract showed prolonged proestrus and estrus phases compared with Group III treated with 1000 mg/kg bodyweight and Group IV (control), whereas the metestrus and the diestrus were significantly longer in Group IV (control) and Group III treated with 1000 mg/kg body weight.

The following phytochemical constituents; carbohydrates, saponins, flavonoids, tannins, alkaloids, steroids and triterpene were detected while anthraquinones were absent in the extract.

**Table 1:** The Phytochemical constituent of the crude leaves extracts of *Rhynchosia sublobata*

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids and Triterpene</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present; - = Absent

**Table 2:** Mean± S.D of the percentage of rats at different phases of estrous cycles at different extract dose levels.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Percentages (Mean± SD) of rats at different phases of the Estrous cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proestrous</td>
</tr>
<tr>
<td>5000</td>
<td>50.07±3.2</td>
</tr>
<tr>
<td>2500</td>
<td>43.73±5.4</td>
</tr>
<tr>
<td>1000</td>
<td>32.03±6.5</td>
</tr>
<tr>
<td>Control</td>
<td>26.03±1.8</td>
</tr>
</tbody>
</table>
The results showed that; Group I treated with 5000 mg/kg body weight and Group II treated with 2500 mg/kg body weight of the extract, produced an irregular pattern of cyclicity or oestrus cycle, which was characterized by significant (P>0.05) increase in the percentage of rats in proestrus and estrus phases of the cycle compared with group IV (control) and Group III treated with 1000 mg/kg bodyweight, which had longer metestrus and diestrus phases.

Discussion

In this study, the variations in the phases of the estrous cycle in the experimental rat groups have shown a dose dependent pattern; with a prolonged proestrus and estrus phases of the cycle in rats treated with 5000 mg/kg, 2500 mg/kg and 1000 mg/kg body weight of the extract respectively when compared with the control group (Figure 1) during the twelve (12) days of observation. This is similar to earlier reports (Anita 1951) and (Marcondes et al. 2002) who demonstrated that; proestrus and the estrus phases were prolonged than the metestrus and the diestrus phases.

There was a significant increase in the duration of proestrus and estrus phases in the treated groups which is suggestive of antifertility effects (Marcondes et al. 2002). Also, (Oluymemi et al. 2008) has reported similar observation.

Phytochemical analysis of *R. sublobata* detects the following constituents: Carbohydrates, Saponins, Flavonoids, Tannins, Alkaloids, Anthraquinones, Steroids and Triterpene. Some of these have been incriminated to cause disorder in oestrus cycle in animals (Farnsworth et al. 1975; Olabiyi et al. 2006).

Also, in a study using *Aspilia Africana* leaf extract (Oluymemi et al. 2008) have detected the presence of saponins and alkaloids. These two compounds have caused the same pattern of cyclicity in Wistar rats; these were also detected in the leaves of *R. sublobata* used in this study.

The significant increase in the duration of the estrus and proestrus phases observed in this study could also be attributed to the steroidal component of the extract. To corroborate this, (Singh 1969) and (Santos et al. 1995) have demonstrated that when female rats are exposed to plant steroidal saponin, they enter into a state of permanent estrus, thereby, increasing the duration of the estrus phase of their cycle.

Similarly, phytoestrogenic plants, which contained saponins and flavonoids are capable of elevating the level of circulating estrogen in the female rats. The high level of estrogen prolongs estrus and proestrus phases of estrous cycle, thereby interfering with fertility (Santos et al. 1995). The disruption in cyclicity due to steroidal saponin on the estrous cycle in the rats has also been reported (Tamura et al. 1997). These phytoestrogenic components of plants have been found to reduce fertility in animals upon continuous administration (Tamura et al. 1997). In our study, the presence of saponins and flavonoids has been detected in the crude aqueous and ethanolic extract of *R. sublobata*. This could be the possible factors that caused disruption of oestrous cycle phases observed in this study. The persistence in the proestrus and estrus phases of the cycle is also an indication of fertility disorder probably caused by the presence of flavonoids, saponin and alkaloid components in *R. sublobata* leaf extract.

Conclusion

It was concluded that the ethanolic extract of a *Rhynchosia Sublobata* exert antifertility effects by affecting phases of oestrous cycle in treated rats. Therefore, pastoralists in Gujba LGA should be cautious when grazing their animals in the field particularly where *R. sublobata* is suspected and or infested; to avoid observed effects of the plant in grazing animals.

References


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GraphPad. 2000. GraphPad InStat version 3.05 for Windows 95, GraphPad Software Inc., San Diego California USA, (www.graphpad.com).


