ANTIFERTILITY EFFECTS OF ABRUS PRECATORIUS ETHANOL SEEDS EXTRACT ON FEMALE RATS

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ABSTRACT

Abrus precatorius, family Fabaceae, is used in many parts of the world as contraceptive and abortifacient. This study was conducted to determine effects on uterine contraction, estrous cycle, serum levels of reproductive hormones, ovarian and uterine weights, and subsequent effects on histology. **Method:** The non-pregnant isolated rat uterus was subjected to the extract to determine effect on contractility. Eighteen regularly cycling female albino rats weighing 130-170g were selected, and divided into three groups of six each; one control receiving normal saline orally 10ml/kg once daily for two weeks while observing the estrous cycle. After two weeks from the last dose animals were examined to see if the cycle was normal. Another group of 18 normally cycling rats was divided as above and given the extracts for two weeks; 24hrs from the last dose blood was collected for hormonal assay. Animals were sacrificed under light ether anesthesia; uteri and ovaries were quickly removed and weighed in a sensitive balance, then preserved in formal saline to prepare histological slides later. **Results:** The extract produced significant uterine contraction. The estrous cycle was extended to 5.07 ± 0.2, and 5.37 ± 0.4 days in the 20 and 40 mg/kg extract groups respectively compared to 4.2 ± 0.2 days in the control group. Proestrus, estrus and diestrus stages were significantly longer (p < 0.05) in both treated groups. The cycles returned to normal after stopping the doses. The serum levels of progesterone were less than those in the control group, however, the reduction was not statistically significant. The weight of the ovaries (p <0.05) and uteri was reduced.
Histopathological study showed fewer and degenerated follicles, and reduced uterine lumen with fewer glands. **Conclusion:** The ethanol seed extract of *Abrus precatorius* causes strong uterine contraction, disrupts the estrous cycle reversibly, and reduces the progesterone levels.

**KEY WORDS:** Antifertility, uterotonic, A. precatorius, progesterone, ovaries.

**INTRODUCTION**

*Abrus precatorius*, family Fabaceae, is a twining herb, with long, pinnate-leafleted feathery leaves. Its flowers are rose to purple in color. It is a legume; the fruits are short pods, containing hard, shiny, scarlet and black seeds. The plant is mostly found in the tropical and subtropical areas of Asia and Africa.

The plant contains many chemical compounds that have been identified, and their structures elucidated. The plant contains alkaloids, flavonoids, phenols, terpinoids, and is rich in protein. The seed of *A. precatorius* is toxic, this is attributed to abrin; a protein constituent of the seed that prevents protein synthesis by inactivating the 26S subunit of the ribosome.

The seeds and other parts of the plant are used traditionally as ornament, contraceptive and abortifacient in parts of central Africa and the Far East. The plant also has hypoglycemic effects. *A. precatorius* has potent anti oxidant activity because of its phenolic and flavonoid constituents. Cytotoxic activity against a variety of cancers e.g. Yoshida sarcoma in vivo and in vitro, human hepatic G2 cells, and Ehrlich Ascites Tumor, and Yoshida Ascites hepatoma were well documented.

The plant’s extract attenuated alcohol induced renal damage, and has antibacterial activity against a range of G+ve and G-ve bacteria.

Research conducted on the *A. precatorius* seeds concluded that the plant suppresses male reproductive functions reversibly. The extract affected oxidative/energy metabolism of cauda epididymis, protein, sialic acid, acid phosphatase and succinic dehydrogenase levels were significantly depleted. Jahan and his group detected an irreversible damage in sperm DNA integrity, thus suggested possible teratogenicity. A study found that methanolic seed extracts of *A. precatorius* caused reversible disruption in oestrus cycle in female rats, completely blocked ovulation, and caused reversible changes in histology.
Medicinal plants continue to be generous sources of effective products that control fertility in both males and females, here we hope verify some of the activities attributed to A. precatorius regarding female fertility, and may be provide a promising contraceptive.

Objectives
Determine the effect of the ethanol seed extract on uterus contraction, estrous cycle, hormonal levels, the weight of reproductive organs and histopathological changes.

MATERIALS AND METHODS
1. Materials
1.1. Plant collection
The seeds were purchased from a local herbalist shop (El Tieman), Omdurman City, Sudan. They were authenticated by a taxonomist, Department of Botany, Faculty of Science, University of Khartoum, and a sample was kept in their herbarium.

1.2. Animals
Female Swiss albino rats weighing 130-170 g were used for this study. The animals were bred and housed in the Faculty of Pharmacy, University of Khartoum animal house, under international standards of animal keep and breed. The animals were given a balanced feed, allowed free access to food and water, living in a light/darkness cycle of 12 hrs approximately.

1.3. Extraction
The seeds were crushed to reduce their size, 140g were placed in soxhelt extractor with 80% ethanol and run for 8 hours, the solvent was evaporated under reduced pressure, and then under room temperature until completely dry. A semi solid dark red material resulted. Yield was 8.6%. For preparing doses the extract was weighed and dissolved in distilled water. Small amounts were usually prepared to avoid storing and probable bacterial growth and deterioration.

2. Methods
2.1. The isolated non-pregnant rat uterus
The uterus was extracted from a freshly killed rat and suspended in De Jalon’s solution at 32°C. The tissue was allowed to adapt, isotonic contractions recorded, acetylcholine was used
as standard. Different doses of the extract were used, and responses recorded. Each dose was repeated three times, the means were used to plot dose response curve and determine ED\textsubscript{50}.

2.2. The estrous cycle

The unstained smear method after Marcondes (2002) \textsuperscript{28} was employed to determine the different stages of the estrous cycle. Smears were prepared by lavage with normal saline, using a plastic pipette. A drop was placed on a slide and examined under light microscope (x10, x40) to identify three main types of cells; epithelial, cornified and leukocytes. Rats that showed regular proestrous, estrous, metestrous (dieoestrus 1) and dieoestrus (dieoestrus 2) for three consecutive cycles were considered regular and included in the experiments.

Eighteen adult female rats, with regular estrous cycles were selected and randomly allocated into three groups. One received normal saline, and two Abrus precarius extract 20 and 40 mg/Kg orally. Doses were given for two weeks and the animals were observed for estrous stages. After two weeks from the last dose estrous cycle was observed in the treated animals to find whether the effect is reversed or not.

2.3. Hormonal assay

Eighteen female rats with regular cycle were randomly divided into three groups similar to the above experiment. The doses were given orally for 15 days. Twenty four hours after the last dose, blood samples were collected onto a plain blood container and allowed to clot. The clotted blood was centrifuged to obtain sera, this was kept frozen until analysis. Levels of estrogen, progesterone, LH and FSH were determined biochemical method and recorded.

2.4. Reproductive organs weight

The animals from the above experiment above were sacrificed; ovaries and uteri were rapidly removed from the animals and weight to the nearest decimal in a sensitive balance.

2.5. Histopathology

Ovaries and uteri were kept in formal saline, then cut and stained in haematoxyylene and eosin. The slides were studied by a pathologist.

2.6. Analysis

The results obtained were presented as mean ± SEM, student t test and ANOVA were performed to analyze differences using the SPSS 17.
RESULTS

Figure 1: the effect of *A. precatorius* on non pregnant uterus

ED50= 1.26x10^-4 g/ml

Table 1: The effect of *A. precatorius* on the estrus cycle and its different cycle stages

<table>
<thead>
<tr>
<th>Group</th>
<th>Cycle length</th>
<th>Stage length days ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Proestrus</td>
</tr>
<tr>
<td>A  Control</td>
<td>4.2 ± 0.2</td>
<td>0.80 ± 0.04</td>
</tr>
<tr>
<td>B  A. precatorius 20mg/Kg</td>
<td>5.07 ± 0.23</td>
<td>0.74 ± 0.03*</td>
</tr>
<tr>
<td>C  A. precatorius 40mg/Kg</td>
<td>5.37 ± 0.38</td>
<td>1.04 ± 0.16*</td>
</tr>
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</table>

n= 6  p< 0.05

Table 2: the effect of *A. precatorius* seed extract on reproductive organs

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<tr>
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<tbody>
<tr>
<td>A  Control</td>
<td>135.75 ± 4.35</td>
<td>150.5 ± 2.25</td>
<td>.11 ± .008</td>
<td>.284 ± 0.08</td>
</tr>
<tr>
<td>B  A. precatorius 20mg/Kg</td>
<td>134.0 ± 9.86</td>
<td>143.0 ± 9.51</td>
<td>.071 ± .008*</td>
<td>.134 ± 0.0178</td>
</tr>
<tr>
<td>C  A. precatorius 40mg/Kg</td>
<td>153.75 ± 4.27</td>
<td>152.25 ± 7.19</td>
<td>.087 ± .002*</td>
<td>.174 ± .063</td>
</tr>
</tbody>
</table>

n=6  *p < 0.05

Table 3: the effect of *A. precatorius* extract on estrogen and progesterone levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Estradiole ng/mL ± SEM</th>
<th>Progesterone ng/mL± SEM</th>
</tr>
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<tbody>
<tr>
<td>A  Control</td>
<td>139.3 ± 20.97</td>
<td>18.76 ± 6.86</td>
</tr>
<tr>
<td>B  A. precatorius 20mg/Kg</td>
<td>144.93 ±3.33</td>
<td>9.16 ± 3.02</td>
</tr>
<tr>
<td>C  A. precatorius 40mg/Kg</td>
<td>142.98 ±29.6</td>
<td>13.25 ± 1.53</td>
</tr>
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</table>

n= 6. Levels of FSH and LH were below 2ng/ml in all groups
Figure 2a: transverse section from a normal ovary H&Ex40.

Figure 2b: transverse section from a normal ovary H&Ex100

Figure 2 a & b show ovaries from control females, with follicles at various stages of development and dense stroma.

Figure 3a: ovaries from rats treated with A. precatorius H &E x 40
Figure 3b: ovaries from rats treated with *A. precatorius* H & E x 100

Figure 3 a&b: ovaries from female rats receiving *A. precatorius*; showing fewer follicles, cystic follicles, loose stroma and haemorrhage.

4a: H &E x 40

4b: H & E x 100

Figure 4 a& b: uterus from the control group showing normal, numerous glands, and dense stroma.
Figure 5 a; H&Ex40

Figure 5b: H&E x 100

Figure 5 a & b: uterus from females treated with A. precatorious, showing hyperplastic gland, fewer glands, loose stroma, reduced lumen and epithilailization.

The extract of *A. precatorius* caused increased frequency and amplitude of uterine contractions, the maximum contraction height was about 80% of that obtained maximally by acetylcholine. ED50 was $1.26 \times 10^{-4}$ g/ml, the steep dose response curve indicates efficacy (figure 1). The interaction between the extract and uterine tissues was revisable; the effect was abolished when the extract washed.

The mean cycle length in the animals employed in the experiments was $4.2 \pm 0.2$ days which is in agreement with Mandle (1951).[29] *A. precatorius*, at both doses caused a statistically
insignificant extension of the estrus cycle length from 4.2 days to 5.07±0.23 in the 20mg/kg group and to 5.37 ±0.38 in the 40mg/kg group. The different phases of the cycle were kept in sequence similar to the normal cycle. All four stages of the estrus cycle were extended. Proestrus, estrus and diestrus elongation was statistically significant (p<0.05). However, the lower dose made slight but significant reduction in proestrus length. Some of the proestrus slides displayed dominance of nucleated epithelial cells with very few or absent cornfield cells, leukocytes were not present; this reflects delayed ova maturation, or anovulation.

After 14 days from the last dose the estrus cycle returned to normal in groups treated with A. precatorius at both concentrations.

A. precatorius elicited a significant reduction in ovarian weight (p<0.05) from 0.11± 0.008g in the control group to 0.071±0.008 and 0.87± 0.002g respectively in the groups taking 20 and 40 mg/Kg of the extract. Similarly, uterine weight was reduced but statistically insignificant, to 0.134±0.01 and 0.174±0.063g compared to 0.284 ± 0.085 in the control group. The reduction in reproductive organs weight might indicate lower levels of gonadotrophins.

Serum levels of estradiol were slightly increased (142, 144 ng/ml, compared to 139 ng/ml in control group) in the animals treated with A. precatorius. Greater reduction was seen in progesterone levels (9.16 , 13.25 ng/ml, compared to 18.76ng/ml in control), yet not statistically significant. However, this was enough to reduce gonadotrophins. The ratio between estrogens: progesterone was affected. Serum levels of FSH and LH were undetectable i.e. below ng/ml in all groups.

Histopathological study of ovarian tissues showed reduced number of follicles, hyperplastic underdeveloped follicles, loose stroma; and some slides showed haemorrhage. Atritic follicles were also present. The uteri slides showed loose stroma, with fewer glands, hyperplastic glands, reduced lumen and reduced epithelial lining.

**DISCUSSION**

The strong uterine contraction caused by A. precatorius activity might indicate that the extract have abortifacient activity; this is in accordance the traditional use as abortifacient 

Many abortifacient plants act through uterotonic action like *Rumex steudelii*  

Increased
uterine motility might also affect implantation, and therefore the plant might have putative antiimplantation activity.

The disruption of the estrous cycle can be caused by both lower or higher levels of estrogen/progesterone. Exogenous estrogen (or estrogen like) cause reduction in gonadotrophins (FSH, LH) through negative feedback mechanisms. The result would be anovulation and reduction in ovarian weight. A. precatorius is rich in alkaloids, flavonoids, phenols, terpinoids, that might have estrogenic activity. This is supported by the fact that FSH and LH levels were undetectable (below 2ng/ml). Aspilia Africana, a medicinal plant rich in saponins, saponin glycosides, steroids, tannins, volatile oils and alkaloids; disrupts estrus cycle, and reduces the duration of proestrus, estrus and metestrus while increasing diestrus\[31\] due to the high phytoestrogen contents. C. longa and C. carvi exhibited gonadotrophin reduction similar A. precatorius due to their contents of estrogen like constituents.\[32\]

The observed ovarian and uterine weight reduction is probably due to reduced gonadotrophins, in turns both ovulation and implantation would be affected. Reduced ovarian weight might indicate suppressed ovulation, this is evident in the histopathology slides that demonstrated reduced follicular development. The reduction in uterine weight designate diminished ability to receive fertilized ova.

Progesterone is secreted from corpus luteum, and is responsible for maintaining the proliferated uterus and, prepare for implantation of the fertilized ova. The reduced progesterone levels might suggest antimplantation activity. In a study by Okoko (2012) similar observations were made, although he used a higher dose (50mg/Kg) for a longer period, he also noted that the changes were reversible.

CONCLUSION

The seed extract of A. precatorius has antifertility effects on female albino rats; it causes uterine contraction, extension of the estrous cycle; significantly increasing proestrus, estrous and diestrus stages. Serum levels of progesterone are reduced although none statistically significant but were able to produce significant ovarian and uterine weight reduction, with marked histological changes.
REFERENCES


27. (Okoko, 2012).


32. (Thakur 2009).