Anti-fertility effect of flower extracts of *Tabernaemontana divaricata* in rats

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[ABSTRACT] **AIM:** To evaluate the anti-fertility effect of methanolic (MeTD) and aqueous (AqTD) flower extracts of *Tabernaemontana divaricata* in rats.  |
**METHODS:** The anti-fertility activity of the extracts was evaluated using two experimental animal models: 1) Estrogenic activity was carried out in immature female rats using ethinyl estradiol as standard. The evaluation parameters includes changes in uterine weight and histopathology of uterus. 2) Anti-implantation and early abortifacient activity was performed in female Wistar rats. The number of implants and resorptions were compared to vehicle control.  |
**RESULTS:** Phytochemical analysis of MeTD and AqTD revealed the presence of carbohydrates, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins. In estrogenic activity, the MeTD and AqTD were offered significant estrogen-like activity at 500 mg·kg⁻¹, p.o. by increasing the uterine weight compared to vehicle control group. In Anti-implantation and early abortifacient activity study, MeTD (500 mg·kg⁻¹, p.o.) showed significant effect and it was evident by decrease in the number of implants and increase in the number of resorptions compared to vehicle control group.  |
**CONCLUSION:** The MeTD at 500 mg·kg⁻¹, p.o. possess significant estrogenic, anti-implantation and early abortifacient activity, while the AqTD at 500 mg·kg⁻¹, p.o. was found to possess significant estrogenic activity and the results are in consistent with the literature reports related to anti-fertility effect of flower extracts of *Tabernaemontana divaricata*.

[KEY WORDS] *Tabernaemontana divaricata*; Estrogenic activity; Anti-fertility effect; Anti-implantation activity; Abortifacient effect


1 Introduction

Family planning has been promoted through several methods of contraception, but due to serious adverse effects produced by the synthetic steroidal contraceptives, now the attention has been focused on indigenous plants for possible contraceptive effect. Although contraceptives containing estrogen and progesterone are effective and popular, risks associated with the synthetic drugs have triggered the need to develop an effective and safe contraceptive drug from medicinal plants. Hence there is a need for searching a suitable product from indigenous medicinal plants that could be effectively used in place of pills¹.

The investigation of plant constituents with anti-fertility properties represents a potential alternative approach to birth control from the existing methods. If an estrogen from a local source could be shown to be active in humans, it would be of great value as a fertility regulating agent. It is noted that anti-fertility agents work by disrupting or desynchronizing pre-ovulatory and pre-implantation events. Anti-fertility activity is often due to estrogenic activity, but can also be due to anti-estrogenic activity. Furthermore, plants can act as anti-fertility agents and these plants can be classified according to their activity profile, such as anti-ovulatory plants, interceptory and abortifacient plants, uterine tonus and uterine stimulants². However many modern medicines are developed through the clues obtained from phytochemicals.

Already several scientific papers have been published related to fertility control from medicinal plants, *Tabernaemontana divaricata* (Linn.) R.Br. is one of such plants that has been advocated as a traditional medicine for family planning. Chewing of seven flowers of the plant, daily for two months after menses, with water in the morning will check the pregnancy for a year and may be continued for longer period for prolonging its effect¹. However, there is a paucity of scientific evidence for its usage as a anti-fertility agent, Henceforth the present study was undertaken to evaluate...
the anti-fertility effect of flower extracts of *Tabernaemontana divaricata* using different experimental models.

## 2 Materials and Methods

### 2.1 Chemicals and drugs

All the solvents used for the extraction process are of laboratory grade and purchased from S.D fine chemicals, Mumbai and Progynon-C (Zydus Cadila (GRem)) were used for the study.

### 2.2 Animals

Female Swiss albino mice (18–22 g), Wistar albino rats 150–200 g and immature female Wistar albino rats of 21–23 days old (40–60 g) were used in this study. They were procured from NIMHANS, Bangalore. The animals were housed in polypropylene cages and acclimatized for ten days under laboratory conditions, i.e.; room temperature of (25 ± 10) °C; relative humidity 45%-55% and a 12 : 12 h light/dark cycle. The animals had free access to standard rat pellet (Gold Mohur Lipton India Ltd), with water supplied *ad libitum* under strict hygienic conditions.

Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any non-specific stress.

The approval of the Institutional Animal Ethical Committee (IAEC) of SCS College of Pharmacy, Harapanahally (Karnataka) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### 2.3 Plant material

Flowers of *Tabernaemontana divaricata* (Linn.) R.Br. were collected from Harapanahalli, Karnataka. And the plant material was authenticated by Prof. K. Prabhu, Department of Pharmacognosy, SCS College of Pharmacy, Harapanahalli. A voucher specimen has been deposited at the museum of the Department.

### 2.4 Preparation of extracts

The flowers of *Tabernaemontana divaricata* were collected and shade dried. The dried flowers were coarse powdered and the powder was successively extracted with petroleum ether (60-80 °C), methanol (64.5-65.5 °C) and distilled water using soxhlet apparatus. The extracts were concentrated using vacuum dryer; the percentage yield of petroleum ether, methanolic and aqueous extracts was found to be 1.8%, 40% and 8% *W/W* respectively.

### 2.5 Preliminary phytochemical screening

Preliminary phytochemical screening was carried out according to standard methods described in practical pharmacognosy by C.K. Kokate[3] and K.R. Khandelwal[4].

### 2.6 Acute oral toxicity

The acute toxicity for MeTD and AqTD was determined in nulliparous and non-pregnant female Swiss albino mice, according to OECD Guideline No. 420 - Fixed dose method[5].

### 2.7 Formulation and drug administration

The MeTD and AqTD were formulated daily just before the administration freshly; In brief required quantity of the extract was weighed and suspended in 0.5% tween 80 and distilled water, the final volume was made up with distilled water, the prepared formulation were administered at a volume of 10 mL·kg⁻¹, p.o. to respective group of animals.

### 2.8 Anti-fertility activity

#### 2.8.1 Estrogenic activity in immature rats[6 -7]

21–23-days old immature female Wistar rats weighing 40–60 g were used. They were divided in to six groups; each consisting of 6 animals each. G-I was served as vehicle control (0.5% Tween 80 in distilled water) received only vehicle; G-II was treated with reference standard ethinyl estradiol 0.02 mg·kg⁻¹ s.c. and G-III & G-IV received aequous extract of *Tabernaemontana divaricata* (AqTD)) 250 and 500 mg·kg⁻¹, p.o. and G-V & G-VI received methanolic extract of *Tabernaemontana divaricata* (MeTD) 250 and 500 mg·kg⁻¹, p.o. respectively for 3 days.

24 h after the last treatment, all the animals were sacrificed by decapitation and uteri were dissected out, cleared off the adhesive tissue, blotted on filter paper and weighed quickly on a sensitive balance. The tissues were fixed in Bouin’s fixative for 24 h, dehydrated in alcohol and embedded in paraffin. The paraffin blocks were sectioned at 6 micron thickness and stained with haematoxyline-eosin solution (H & E Stain) for histological observations.

#### 2.8.2 Anti-implantation and early abortifacient activity in rats[8]

Female wistar albino rats of 150–200 g were used to assess anti-implantation and early abortifacient activity. Vaginal smears from each rat was monitored daily during acclimatization. Only the rats with normal oestrous cycles were selected for the experiment.

Female rats of proestrus phase were kept with male rats of proven fertility for mating in a ratio of 2 : 1. On the next morning females were examined for evidence of copulation. The animals exhibiting thick clumps of spermatozoa in vaginal smears were separated from male partner. The day when spermatozoa were detected in the vaginal smear was considered as day-1 of gestation.

The separated pregnant rats were divided into five groups of six rats each. G-I was served as vehicle control received only vehicle (0.5% Tween 80 in distilled water); G-II was treated with reference standard ethinyl estradiol 0.02 mg·kg⁻¹ s.c.; G-III & G-IV received aequous extract of *Tabernaemontana divaricata* (AqTD) 250 and 500 mg·kg⁻¹, p.o. and G-V & G-VI received methanolic extract of *Tabernaemontana divaricata* (MeTD) 250 and 500 mg·kg⁻¹, p.o. respectively.

The extracts were administered orally from day-1 to day-7 of gestation. On the 10th day laparotomy was carried out under light ether anesthesia in sterile conditions. The uteri
were examined to determine the number of implantation sites; the number of corpora lutea in ovaries were recorded. The abdomen was sutured and the animals were left in cages. The drugs were administered orally again for 3 days (day 14-16). On the 18\textsuperscript{th} day laprotomy was carried out again for evaluating the early abortifacient activity.

The percentages of anti-implantation and early abortifacient activities were calculated using formula given in Equation 1 and 2. The sum total of anti-implantation and early abortifacient activity gives percentage anti-fertility activity of the extract (Equation 3).

\textbf{Equation 1}
\[
\text{\% Anti-implantation activity} = \frac{100 \times (\text{No. of Corpora lutea} - \text{No. of Implantations})}{\text{No. of Corpora lutea}}
\]

\textbf{Equation 2}
\[
\text{\% Abortifacient activity} = \left(\frac{\text{No. of resorptions}}{\text{No. of Corpora lutea}}\right) \times 100
\]

\textbf{Equation 3}
\[
\text{\% Anti-fertility activity} = \text{\% Anti-implantation activity} + \text{\% Abortifacient activity}
\]

\textbf{Statistical analysis}
Values were expressed as $\bar{x} \pm s$ from 6 animals. Statistical difference in the mean will be analyzed using one way ANOVA followed by Turkey’s multiple comparison tests $P < 0.05$ was considered as statistically significant.

\section{Results}
\subsection{Phytochemical analysis}
Preliminary phytochemical analysis of the extracts revealed the presence of carbohydrates, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins in both MeTD and AqTD.

\subsection{Acute oral toxicity}
No morbidity and mortality were detected till 2000 mg\textsuperscript{•}kg\textsuperscript{-1}, p.o. for both MeTD and AqTD, hence MeTD and AqTD were considered to be safe till 2 000 mg\textsuperscript{•}kg\textsuperscript{-1}, p.o.

\subsection{Anti-fertility activity}
\subsubsection{Estrogenic activity in immature rats}
Treatment with MeTD (250 and 500 mg\textsuperscript{•}kg\textsuperscript{-1}, p.o.) and AqTD (250 and 500 mg\textsuperscript{•}kg\textsuperscript{-1}, p.o.) had showed significant increase in uterine weight in a dose-dependent manner compared to vehicle control. The estrogenic effect of AqTD at 500 mg\textsuperscript{•}kg\textsuperscript{-1}, p.o. was comparable with reference standard ethynil estradiol (0.02 mg\textsuperscript{•}kg\textsuperscript{-1}, p.o.). Furthermore, the MeTD at 500 mg\textsuperscript{•}kg\textsuperscript{-1} offered more potent estrogenic activity than the reference standard ethynil estradiol (Fig. 1). The results obtained were also correlated and supported by the histopathological findings, where the MeTD (500 mg\textsuperscript{•}kg\textsuperscript{-1}, p.o.) showed significant increase in the height of luminal epithelium, loose and edemators stroma with stimulated uterine glands; while the AqTD (500 mg\textsuperscript{•}kg\textsuperscript{-1}, p.o.) showed moderate increase in the height of luminal epithelium with stimulated uterine glands (Fig. 2 to 7).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Estrogenic activity \textit{Tabernaemontana divaricata} flowers extracts in immature rats}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Photomicrograph showing section of uterus indicating surface epithelium with no secretory activity (Control group) HE 300\times}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Photomicrograph showing section of uterus indicating increase in height of luminal epithelium (Ethynil Estradiol) HE 300\times}
\end{figure}

\subsection{Anti-implantation and abortifacient activity}
The anti-implantation activity is expressed as the percentage decrease in the number of implantations in the uteri on day-10 of pregnancy and the number of resorbed implants from the existing number of implants will be recorded on day-18 for evaluating the early abortifacient activity.
Fig. 4  Photomicrograph showing section of uterus indicating increase in height of luminal epithelium (MeTD-250 mg·kg⁻¹) HE 300X

Fig. 5  Photomicrograph showing section of uterus indicating increase in height of luminal epithelium, loose and edematous stroma with stimulated uterine glands (MeTD-500 mg·kg⁻¹) HE 300X

The MeTD and AqTD have offered significant and dependent anti-implantation and early abortifacient activity by decreasing the number of implantation sites and also showed significant resorbtion of the existing implants compared to vehicle control.

Table 1  Effect of Tabernaemontana divaricata flower extracts on anti-implantation and early abortifacient activity in rats ( x ± s, n = 6)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Antiimplantation activity</th>
<th>% Early Abortifacient activity</th>
<th>% Anti-fertility activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AqTD -250 mg·kg⁻¹, p.o.</td>
<td>24.28 ± 1.23</td>
<td>2.85 ± 0.59**</td>
<td>27.13 ± 1.41</td>
</tr>
<tr>
<td>AqTD -500 mg·kg⁻¹, p.o.</td>
<td>41.42 ± 2.41</td>
<td>5.71 ± 2.54***</td>
<td>47.13 ± 2.41</td>
</tr>
<tr>
<td>MeTD-250 mg·kg⁻¹, p.o.</td>
<td>30.30 ± 1.05</td>
<td>3.03 ± 0.42**</td>
<td>33.33 ± 1.01</td>
</tr>
<tr>
<td>MeTD-500 mg·kg⁻¹, p.o.</td>
<td>63.33 ± 3.10</td>
<td>10.00 ± 4.15***</td>
<td>73.33 ± 3.74</td>
</tr>
</tbody>
</table>

** p < 0.01, *** p < 0.001 vs vehicle control

4  Discussion

It is well known that estrogenic substances inhibit pregnancy by suppressing the level of both follicular stimulating hormone (FSH) and luteinizing hormone (LH) which in turn prevent the implantation. Estrogen and progesterone are the hormones responsible for the histologic and functional modifications of female genital tract. The exogenous administration of physiological doses of estrogen stimulated the histoarchitecture of uterus in sexually immature rats[10]. Any compound possessing estrogenic activity may exhibit anti-fertility activity by suppressing the gonadotrophin secretion along with consequent inhibition of ovulation[10].

In immature female rats both MeTD and AqTD have exhibited estrogenic activity in a dose-dependent manner. The extracts may be acting through imbalancing the endogenous estrogen and progesterone levels; the loss of implantation caused by the extracts may be due to their anti-zygotic, blastocytotoxic or anti-implantation activity[11]. The histopathology of animals treated with MeTD (500 mg·kg⁻¹, p.o.)
showed increased height of luminal epithelium with hyperplasia, loose and edematous stroma with stimulated uterine glands. While the animals treated with AqTD (500 mg·kg⁻¹, p.o.) showed increased height of luminal epithelium with stimulated uterine glands.

In literature reports, the ingestion of 200, 400 and 800 mg·kg⁻¹ of ethanolic extract of Salvia fruticosa from day-1 to day-6 of pregnancy did not cause pregnancy failure, but reduced the number of viable fetuses and increased the number of resorptions in female pregnant rats[11]. Vasicine, isolated from Adathoda vasica showed potent abortifacient and uterotoxic effects in guinea pigs[12]. Flavonoids isolated from Striga lutea and Striga orobanchioides possessed strong estrogenic and anti-fertility properties[13-14].

Hence the anti-fertility activity of the MeTD and AqTD may be mainly due to their estrogenic activity. The phytochemical constituents such as isoflavones along with coumentans (also flavonoids) and lignans belong to a class of substances known as non-steroidal phytoestrogens, and they produce infertility in animals[15]. In addition, it has also been proved that several commonly occurring flavonoids mimic the biological effects of 17β-estradiol by virtue of their ability to bind and activate the nuclear estrogen receptors[16].

The phytochemical tests revealed that, both MeTD and AqTD are rich in flavonoids. Hence it is thought the high flavonoid content of the MeTD and AqTD may be responsible for significant anti-fertility activity against the selected experimental models and further studies are needed to characterize and isolate the component responsible for the anti-fertility activity.

5 Conclusion

With these preliminary results we can conclude that the MeTD and AqTD showed significant anti-fertility activity by means of potent estrogenic, anti-implantation and early abortifacient activities in a dose-dependent manner.

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References