Histology and Teratology of Uterus and Embryo of Mice Treated With Mensifort, an Indigenous Abortifacient Drug

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Abstract

Mensifort, an indigenous abortifacient drug contain a combination of various compounds. The drug was administered intraperitoneally from Day 3 to Day 16 of gestation and 15 Days and 30 Days prior to mating at a dose rate of 300mg/ kg body weight. The drug induced significant foetotoxic effect in mouse during pre- and post- fertilization stages. The histioarchitecture of ovary and uterus in the treated animals showed the presence of hypertropic ovary and the uterus was found to be haemorphagic. The teratologic assessment showed various skeletal anomalies and decrease in body weight, sex ratio and the number of foetuses born to pregnant mice. The drug was observed to increase a higher percentage of dead foetuses and resorption index in the pregnant mice.

Keywords: Abortifacient; Pre- and Post-implantation; Resorption; Haemorphagic; Teratologic; Hypertrophic; Aborted foetuses

Introduction

Global Family planning has been promoted through several methods of contraception to check the rapid increase in population in developed and under developed countries. Serious adverse effect were produced by synthetic steroidal drugs (Bingel and Benoit, 1973 ; Farnsworth et al., 1975 ; WHO Report, 1981). This has warranted to focus the attention on indigenous plants for possible contraceptive effect. Therefore, the formulation of cheaper, affordable and effective contraceptives like Mensifort was evolved to reduce the toxicity of the synthetic drugs. The available scientific information is not sufficient to understand the abortifacient activity. Sometimes the usage of the drug may fail to abort the foetuses (Gopalakrishnan and Rajasekara setty, 1978). Hence the present work was undertaken to evaluate the drug during pre- and post- fertilization and the effect of the drug on the mothers as well as embryos.

Materials and Methods

Experimental Drug

Mensifort is a siddha preparatory medicine available in capsule form. It is a common commercial abortifacient drug. Each capsule contains, Annabethi sentharam, Peganum harmoda, Aristoloescia bracteata, Diosorea eexulentus, Crotolaria juncea, Hibiscus cannabinus, Aloe litterolis, Claviceps purpurea, Balsamodendron and Cocculus hirsutus as ingredients in equal parts. The drug was obtained from M/S. Maragathom Co., Devakottai, Tamil Nadu, India.

Experimental animal

Three months old and weighing 25g to 27g of mouse (Mus musculus) of Swiss albino strain was procured from the Central Animal Facility, Indian Institute of Science, Bangalore. The experiments were carried out at controlled temperature under pathogen free condition, pellet diet and water was provided ad libitum. The Mensifort powder is nicely triturated with distilled water until a homogenous suspension is obtained and 0.5ml of the suspension at a dose rate of 300 mg/ kg body weight was injected intraperitoneally into mouse during various periods of treatment. A parallele solvent and a negative control maintained with distilled water and 1% carboxy methyl cellulose (CMC) respectively.

Experimental Design

The pregnant animals were separated into A, B, C, D and E groups after mating them in the ratio 1:1. The groups A and E formed the distilled water and CMC controls and the other groups formed the drug treated animals. The test drug was injected at varying periods of gestation (D3 – D8, D6 – D9 and D10 – D16). Each pregnant animal received a total dose of the compound amounting to 90mg, 60mg and 1.050mg respectively at varying periods. The pre- fertilization experiments were carried out by injecting 0.5ml of the drug at a dose rate of 300mg / kg body weight. The animals were allowed to mate after giving 30 injections alternatively. The female animals were separated and reared after observing vaginal plugs. On Day 20 of gestation the pregnant animals were dissected and either uterus was analysed histologically. The teratological analysis was carried out in the foetuses born to treated and control animals of various groups during pre- and post- fertilization.

Methods for Histological and Teratological Study

The microtome sections of 6 microns of the uterine tissue was stained in

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hematoxylin and counter stained by eosin. The foetuses born to various groups of the experiments were analysed according to the procedure described by Wilson (1965) and Gupta et al., (1978). They were stained in Alizarin Red. Statistical analysis of the foetuses was carried out (Zar, 1974).

**Results**

The histoarchitecture of the pre-fertilized uterine tissue showed a greater reduction in the size and number of ovaries (Fig. 1d). The myometrium was thin showing haemorphagic condition (Fig. 2c) and ovary was hypertrophic (Fig. 2b). The cross-section of the uterus treated animals of D10-D16 of pregnancy showed significant changed like implanted sites and unorganised embryo (Fig. 1c). The foetuses were not well organized with organ systems in all treatments. The endometrium of treated animals showed a marked enlargement of the stroma with large amounts of cytoplasm and with haemorphagic tips (Fig. 1a & b). Peg like endometrium with haemorphagic tips and haemorphagic myometrium were observed in the uterine tissue of D6-D9 treatment (Fig. 2a & c). The myometrium was observed to be normal in control animals (Fig. 2d).

The results of the teratological assessment of the test drug in mice treated during various periods of gestation are summarised in the tables 1-3 and the skeletal abnormalities of the foetuses are shown in Fig 3 (a-b). Mensifort treated animals of pre- and post fertilization staged showed equal proportions of death and resorption of foetuses. Diverse sex ratio was recorded in the offspring born to pregnant mice that received the drug during different days of gestation (Table 1). A significant decrease in the foetal weight (p < 0.05) was observed in the animals treated during pre- and post-implantation stages (Table 1-3). Mensifort treated groups of animals showed a higher frequency (35.61%) of pre-implantation loss of eggs and dead implants. The litters born to mice treated with the drug during pre- and post-implantation staged showed varying degrees of skeletal anomalies as forked ribs, displaced ribs and missing ribs (Fig. 3).

**Figure 1.** T.S of uterus and ovary of mice treated with mensifort, (a) Pregnant mice D6 – D9 treatment showing Peg like endometrium with haemorphagic tips, (b) Uterus of pregnant mice showing haemorphagic islands, (c) Uterus showing implanted sites with unorganised embryo of D10 – D16 treatment, (d) T.S of Ovary of a treated animal showing small ovaries and mucous

**Figure 2.** T.S of uterus and ovary of treated and control mice. (a) Peg like hypertrophied endometrium with haemorphagic tips of D6 – D9 treatment, (b) Hypertrophied ovary, (c) Uterus showing haemorphagic myometrium, (d) Normal endo and myometrium of Carboxy Methyl Cellulose treated mice (Control).
The foetuses and uterus of treated pregnant and non pregnant animals were histologically and teratologically assessed. The endometrial changes in the pre- and post- treated animals were recorded in the present study. A similar effect was reported to be induced in female albino rats treated with the plant extract of *Plumera rebra* L. (Dimesh Dabhadhakar and Varsha Zade, 2012). The probable interference of the drug with the mitotic division of the foetuses, chemical insults both before and after the implantation process can result in pre- and post- embryonic loss (Elbetieha et al., 2000). The decrease in the number of foetuses and body weight was observed during pre- and post fertilization treatments in the present research work. Similar observation was reported by Shibash et al., 2006, following the administration of methanolic extract of *Achyranthus aspera* L, leaves to pregnant rats. The presence of alkaloids, steroids, phenolic and saponins of the plant component of the drug might be responsible for the possible effects of the present observation in mice treated with mensifort. The foetal resorption observed in the present analysis suggest that interruption of the pregnancy occurred after the implantation of the foetus (Elbetieha et al., 2000). The results observed in the present study on the histology of follicular growth of ovaries coincides with the observations of Solomon et al., (2010). The endometrial changes observed in the present analysis in the animals treated with the drug during pre- and post- fertilization is in agreement with the results of Prakash (1979).

The present observation on the adverse effect on fertility index is in agreement with the reports on the abortifacient efficiency of Indigofera trifoliate leaves extract on female albino rats (Dimesh Dabhadhakar and Varsha Zade, 2013). It was suggested that the estrogenic activity might be responsible for foetal resorption and decrease in body weight of the implantation process can result in pre- and post- embryonic loss (Elbetieha et al., 2000). The results observed in the present study on the histology of follicular growth of ovaries coincides with the observations of Solomon et al., (2010). The endometrial changes observed in the present analysis in the animals treated with the drug during pre- and post- fertilization is in agreement with the results of Prakash (1979).

The skeletal malformations also in agreement with the earlier reports. The skeletal malformations born litters (Pradeepa be responsible for foetal resorption and decrease in body weight of the endometrial changes observed in the present analysis in the animals treated with mensifort. The foetal resorption observed in the present analysis suggest that interruption of the pregnancy occurred after the implantation of the foetus (Elbetieha et al., 2000). The results observed in the present study on the histology of follicular growth of ovaries coincides with the observations of Solomon et al., (2010). The endometrial changes observed in the present analysis in the animals treated with the drug during pre- and post- fertilization is in agreement with the results of Prakash (1979).

### Table 1. Teratological Assessment of Foetuses Born To Pregnant Mice Treated With Single Dose of Abortifacient Compounds At Different Periods Of Gestation

<table>
<thead>
<tr>
<th>Name of Chemical Compound</th>
<th>Dose mg/kg body wt</th>
<th>Period of Treatment</th>
<th>No. Of Foetuses Examined</th>
<th>Sex Ratio* F/100M</th>
<th>Body wt. (gms) X ± SD</th>
<th>Head length (cm) X ± SD</th>
<th>Head width (cm) X ± SD</th>
<th>Body length (cm) X ± SD</th>
<th>Tail length (cm) X ± SD</th>
<th>Hind limb length (cm) X ± SD</th>
<th>Fore limb length (cm) X ± SD</th>
<th>Percentage of foetuses showing physical and skeletal abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (D.W)</td>
<td>0</td>
<td>0</td>
<td>51</td>
<td>95.83</td>
<td>1.76 ± 0.09</td>
<td>1.30 ± 0.13</td>
<td>1.07 ± 0.09</td>
<td>2.76 ± 0.28</td>
<td>1.58 ± 0.20</td>
<td>1.21 ± 0.15</td>
<td>1.30 ± 0.21</td>
<td>0.0</td>
</tr>
<tr>
<td>Mensifort</td>
<td>300</td>
<td>D3 – D8 D6 – D9 D10 – D16</td>
<td>24, 17, 29</td>
<td>117.64 114.28 95.00</td>
<td>1.23 ± 0.56</td>
<td>1.05 ± 0.07</td>
<td>0.91 ± 0.04</td>
<td>2.39 ± 0.09</td>
<td>1.04 ± 0.05</td>
<td>0.93 ± 0.04</td>
<td>0.83 ± 0.03</td>
<td>8.00</td>
</tr>
<tr>
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</tr>
<tr>
<td>Carboxy Methyl Cellulose</td>
<td>1</td>
<td>D3 – D8 D6 – D9 D10 – D16</td>
<td>30, 35, 18</td>
<td>82.14 91.17 93.33</td>
<td>1.51 ± 0.36</td>
<td>1.05 ± 0.06</td>
<td>0.94 ± 0.08</td>
<td>2.51 ± 0.15</td>
<td>1.08 ± 0.10</td>
<td>0.96 ± 0.16</td>
<td>0.84 ± 0.16</td>
<td>6.66</td>
</tr>
</tbody>
</table>

DW : Distilled Water    D : Day of Gestation    X : Mean    SD : Standard Deviation    * Females per 100 Males
Table 2. Teratological Assessment of Foetuses Born to Female Mice Treated with Abrine, Embelin and Mensifort with a single dose for 15 Days prior to Mating

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Dose mg/kg body wt</th>
<th>Number of Foetuses</th>
<th>Fetus weight examined X ± SD</th>
<th>Head length (cm) X ± SD</th>
<th>Head width (cm) X ± SD</th>
<th>Body length (cm) X ± SD</th>
<th>Tail length (cm) X ± SD</th>
<th>Hind Limb length (cm) X ± SD</th>
<th>Fore limb length (cm) X ± SD</th>
<th>Percentage of foetuses showing physical and skeletal abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control [Distilled Water]</td>
<td>0</td>
<td>51</td>
<td>1.76 ± 0.06</td>
<td>1.30 ± 0.13</td>
<td>1.07 ± 0.09</td>
<td>2.76 ± 0.28</td>
<td>1.58 ± 0.20</td>
<td>1.21 ± 0.15</td>
<td>1.30 ± 0.21</td>
<td>0.0</td>
</tr>
<tr>
<td>Solvent Control [Carboxymethyl cellulose]</td>
<td>1%</td>
<td>31</td>
<td>1.40 ± 0.04</td>
<td>1.15 ± 0.05</td>
<td>1.03 ± 0.04</td>
<td>2.68 ± 0.08</td>
<td>1.30 ± 0.09</td>
<td>1.11 ± 0.03</td>
<td>1.00 ± 0.03</td>
<td>3.22</td>
</tr>
<tr>
<td>Mensifort</td>
<td>300</td>
<td>23</td>
<td>1.34 ± 1.02</td>
<td>1.10 ± 0.07</td>
<td>0.97 ± 0.06</td>
<td>2.59 ± 0.08</td>
<td>1.15 ± 0.05</td>
<td>1.08 ± 0.04</td>
<td>0.96 ± 0.05</td>
<td>0.0</td>
</tr>
</tbody>
</table>

X : Mean  
SD : Standard Deviation

Table 3. Teratological Assessment of Foetuses Born to Male Mice Treated with Abrine, Embelin and Mensifort with a single dose for 30 Days prior to Mating

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Dose mg/kg body wt</th>
<th>Number of Foetuses</th>
<th>Fetus weight examined X ± SD</th>
<th>Head length (cm) X ± SD</th>
<th>Head width (cm) X ± SD</th>
<th>Body length (cm) X ± SD</th>
<th>Tail length (cm) X ± SD</th>
<th>Hind Limb length (cm) X ± SD</th>
<th>Fore limb length (cm) X ± SD</th>
<th>Percentage of foetuses showing physical and skeletal abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control [Distilled Water]</td>
<td>0</td>
<td>51</td>
<td>1.76 ± 0.06</td>
<td>1.30 ± 0.13</td>
<td>1.07 ± 0.09</td>
<td>2.76 ± 0.28</td>
<td>1.58 ± 0.20</td>
<td>1.21 ± 0.15</td>
<td>1.30 ± 0.21</td>
<td>0.0</td>
</tr>
<tr>
<td>Solvent Control [Carboxymethyl cellulose]</td>
<td>1%</td>
<td>21</td>
<td>1.39 ± 0.04</td>
<td>1.00 ± 0.07</td>
<td>0.93 ± 0.04</td>
<td>2.67 ± 0.21</td>
<td>1.12 ± 0.07</td>
<td>1.05 ± 0.05</td>
<td>0.98 ± 0.06</td>
<td>0.0</td>
</tr>
<tr>
<td>Mensifort</td>
<td>300</td>
<td>16</td>
<td>1.33 ± 0.06</td>
<td>1.14 ± 0.06</td>
<td>1.01 ± 0.03</td>
<td>2.56 ± 0.08</td>
<td>1.13 ± 0.47</td>
<td>1.07 ± 0.06</td>
<td>0.96 ± 0.07</td>
<td>0.0</td>
</tr>
</tbody>
</table>

X : Mean  
SD : Standard Deviation

observed in the present study might be possible due to infiltration of the enzyme level namely carbonic anhydrase, as reported by Maya Gosh et al., (1995).

In conclusion, the experimental drug possesses abortifacient activity. The study may prove to be an effective and safe alternative remedy for contraception. There is need to study the bioactive principles of the components of the plant compounds of the drug.

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