TOXICOLOGY OF LAPACHOL IN RATS: EMBRYOLETHALITY

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ABSTRACT

Lapachol is a naphthoquinone with therapeutic potential against enterovirus, Chagas disease and is also used as an antimalarial and antiinflamatory agent. In order to study teratogenic potential of Lapachol, pregnant Wistar rats were treated with 0.5 ml of distilled water (control group); 0.5 ml of hydroalcoholic solution (vehicle group) and 10 mg of Lapachol in 0.5 ml of hydroalcoholic solution (treated group) by oral gavage from the 8th to the 12th day of pregnancy. The following variables were observed: maternal body weight on days 1, 6, 15 and 21 and food intake on days 2, 6, 15 and 21 of pregnancy. The number of live and dead fetuses and the sites of resorptions were counted. The ovaries were weighed and the corpora lutea were counted. Data were analyzed by ANOVA-one way, Dunnett test and the chi square test. Significance level test $\alpha = 0.05$. Results have shown that mothers were unaffected but there were a 99.2% of fetus mortality, indicative of a strong abortifacient effect of Lapachol in rats.

Key words: Lapachol, embryotoxicity, rat.

INTRODUCTION

Lapachol is a naphthoquinone (2-hydroxy-3-(3-methyl-2 butenil)-1,4 naphthoquinone), extracted from Pau d’arco (Family Bignoneaceae) (Santana et al., 1968). A great spectra of therapeutic activities have been attributed to Lapachol or its derivatives such as prevention of cercarial skin penetration of Schistosoma mansoni (Pinto et al., 1977); trypa-

RESUMO

Toxicologia do Lapachol em ratas: embriotoxicidade

O Lapachol é uma naftoquinona com potencial terapêutico contra enterovirus, doença de Chagas e como antimalárico. Para estudar o potencial embriotóxico do Lapachol, ratas originalmente Wistar, grávidas, foram tratadas com 0,5 ml de água destilada (grupo controle); 0,5 ml de solução hidroalcoólica (grupo veículo) e 10 mg de Lapachol em 0,5 ml de solução hidroalcoólica (grupo tratado) por gavage, do 8o ao 12o dia de gestação. As seguintes variáveis foram observadas: peso corporal materno nos dias 1, 6, 15 e 21, consumo de ração nos dias 2, 6, 15 e 21 de gestação. O número de fetos vivos e mortos e os locais de reabsorção foram contados. Os ovários foram pesados e os corpos lúteos, contados. Os dados foram analisados por ANOVA “one way”, teste de Dunnet e Qui-quadrado. O nível de significância dos testes foi $\alpha = 0.05$. Os resultados mostram que as mães não foram afetadas, mas ocorreram 99,2% de mortes fetais, o que indica um potente efeito embriotóxico do Lapachol para o rato.

Palavras-chave: Lapachol, embriotoxicidade, rato.
plastic agents (Santana et al., 1968; Rao, 1974; Linardi et al., 1975); antimalarial against erythrocytic stages of *Plasmodium falciparum* (Carvalho et al., 1988) and use against enteroviruses (Pinto et al., 1987).

In spite of these therapeutic possibilities, only a few papers have been published about the toxicology of Lapachol (Santana et al., 1968; Morrison et al., 1970; Austin, 1974). So it seems important to screening the toxic effect on embryo after administration of Lapachol to rats as a part of the studies on the toxicology of this phytoterapic.

**MATERIAL AND METHODS**

Three-month old female Wistar rats, weighing 175 ± 10 g, obtained from the Centro de Biologia da Reprodução vivarium were maintained in temperature-controlled quarters with a light – dark period of 12:12 hs. Food and water were available *ad libitum*.

Female rats were caged with fertile male. The following day was designated day 1 of pregnancy if spermatozoa or a seminal plug was present in the vagina.

Inseminated females were randomly assigned to one of the following groups: control, vehicle or treated.

Animals of control group were treated with 0.5 ml of distilled water; vehicle group was treated with 0.5 ml of hydroalcoholic solution and the treated one received 10 mg of 0.5 ml of Lapachol hydroalcoholic solution. All treatments were done by oral gavage from the 8th to the 12th day of pregnancy – period of greater sensibility to teratogens in rats (Manson & Kang, 1994).

The dose of Lapachol was calculated on the basis of the therapeutic doses given to humans (25 mg/kg), and the DL-50 which was 1.600 mg/kg of rat body weight (Santana et al., 1968).

In order to observe maternal toxic effect, clinical criteria were adopted (Manson & Kang, 1994) and the following variables were collected: maternal body weight on days 1, 6, 15 and 21 of pregnancy; food intake on days 2, 6, 15 and 21 of pregnancy. Locomotor activities, piloerection, vaginal bleeding and any other unusual behavior were also observed.

Rats were killed on day 21 of pregnancy by an overdose of ether inhalation. The uterus of each animal was exposed and the number of live and dead fetuses were counted.

The sites of resorption were counted and recorded. Live and dead fetuses were examined to detect external malformations.

The ovaries were weighed and the *corpora lutea* were counted.

Statistical analysis were performed using ANOVA-one way Dunnett test and the chi square test to evaluate continuous and discontinuous data, respectively. Significance level test was *α* = 0.05.

The project was approved by CBR ethical committee.

**RESULTS**

Rats of all experimental groups had no alteration of locomotor activities and did not present piloerection. There were no maternal deaths and the food intake was similar among the groups (Table 1).

No maternal body weight loss was observed during the gestation period (Table 2).

Mean of implants and corpora lutea were similar among the experimental groups (Table 3).

The implantation index (total implantation/total corpora lutea × 100) was 89.8%, 91.8% and 95.4%, in the control, vehicle and Lapachol treated groups, respectively. There was no significant difference among the groups.

There were 2%; 0.99% and 99.2% of resorptions and a mean of 10 ± 2 (14); 11 ± 2 (9) and 1.0 live fetus in the control, vehicle and lapachol treated groups, respectively. There was a high significant difference among treated, control and vehicle groups (p < 0.01).

The live fetus had half the weigh of control and vehicle fetuses.

**DISCUSSION AND CONCLUSIONS**

Since no clinical signs of maternal toxicity were observed, except for the food intake reduction in the Lapachol treated rats, it is possible to assume that Lapachol was not toxic to the mothers considering the dose used (Manson & Kang, 1994).

The reproductive performance of mothers can be considered normal since the implantation index, the mean of implants and corpora lutea were similar in all experimental groups.
The conception index correlates the implantation site and the number of corpora lutea (Ford, 1982) – the higher the index the greater the number of ovulation that results in oocytes fertilization and in blastocysts implantation. When the difference between corpora lutea and implantation sites is greater than 10% there is a possibility of reproductive failure (Wilson, 1980).

The development from implanted blastocyst ongoing is coordinated by a sum of events depending mostly on the progesterone secreted by corpora lutea.

The blood concentration level of progesterone was correlated to the number and volume of corpora lutea (Kato et al., 1979), so it is reasonable to assume that progesterone level can be normal since the mean number of corpora lutea was similar among experimental groups.

Taken together, the data above mentioned suggest that the Lapachol treatment did not cause general or reproductive maternal toxicity.

Except for one mother Lapachol treated that had one live fetus, all the others presented 99% of resorbed fetus. Since resorption is an **in situ** autolyse of embryos or fetuses (Kalter, 1980) it is possible to assume that Lapachol is very toxic to the embryos during the early organogenic period. As mothers were unaffected, there was a high incidence of fetal mortality and the single live fetus was 50% lighter than the control ones, it is possible

### TABLE 1
Food intake in control, vehicle and Lapachol (10 mg/rat) treated pregnant rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Food intake (g)/days of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>18.77 ± 5.37 (14)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>15.78 ± 1.03 (9)</td>
</tr>
<tr>
<td>Lapachol</td>
<td>14.50 ± 3.32 (11)</td>
</tr>
</tbody>
</table>

Results are expressed in Mean ± SD (n). * p = 0.020.

### TABLE 2
Body weight (g) in control, vehicle and Lapachol (10 mg/rat) treated pregnant rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Maternal weight (g)/days of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>171.54 ± 12.19 (14)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>182.83 ± 11.66 (9)</td>
</tr>
<tr>
<td>Lapachol</td>
<td>177.17 ± 14.16 (11)</td>
</tr>
</tbody>
</table>

Results are expressed in Mean ± SD (n). * The weight was obtained after uterus, ovaries and conceptuses were removed.

### TABLE 3
Mean of corpora lutea and implants in control, vehicle and Lapachol (10 mg/rat) treated pregnant rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Corpora lutea</th>
<th>Implants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12 ± 1 (14)</td>
<td>11 ± 2 (14)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>12 ± 1 (9)</td>
<td>11 ± 2 (9)</td>
</tr>
<tr>
<td>Lapachol</td>
<td>12 ± 1 (11)</td>
<td>11 ± 1 (11)</td>
</tr>
</tbody>
</table>

Results are expressed in Mean ± SD (n).
to assume that the phytoterapic is embryocid and it is necessary to further evaluate the possibility of lower doses be teratogenic.

In conclusion, in spite of the great therapeutic potentiality of Lapachol it is necessary to evaluate its teratogenic potential and other toxic effects carefully before the clinical use in humans because it is a strong abortifacient in rats and the possibility of it having the same effect in human cannot be discarded.

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REFERENCES


