THE EFFECT OF THE ECHINACEA EXTRACT IN INTOXICATION WITH COPPER ON CHICK EMBRYO

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SUMMARY

In the study realized we observed one by one the effects of the copper, of the Echinacea extract and both of the copper and the Echinacea extract in the case of chick embryo. Several sets of eggs have been taken into consideration to which we applied the technique of windows make. So that we have access to the embryos and that we observe their evolution during the experiment. The observation of the experiment showed a powerful teratogenic and embryotoxic effect of the copper, no effect of the Echinacea extract, but in the case of the copper administration together with the extract we couldn’t notice a decrease of the copper effects.

Keywords: Echinacea extract, copper, embryotoxicity, teratogenic

INTRODUCTION

Knowing from the specialized literature about the immunostimulatory and potential antioxidant effect of the Echinacea [1, 2, 3], this paper has the purpose to present the results of the experiment that have been obtained from the study of the effect of the Echinacea extract in the intoxications with copper.

For the accomplishment of this study there have been used hen embryos which
revealed the effects. The sets of eggs have been put into the incubator, windows made, colored and injected in caudal area under embryo one by one with CuCl₂, Echinacea extract, CuCl₂ and Echinacea extract, the effects on each set of eggs have been observed through daily notes about the embryos during a period of a week.

The interpretation of the experimental dates obtained from the observations on the set of eggs taken into consideration, shows a powerful teratogenic and embryotoxic effect of the copper on the chick embryos. As for the antioxidant effect of the Echinacea extract the result wasn’t the one we expected. There wasn’t any decrease of the copper effect after the injection with Echinacea solution.

**Materials and Methods**

**Materials:**
- fertilized hen eggs
- incubator, small trays for eggs, tools for windows make
- binocular glass for observations
- red neutral coloring solution 0.2%
- CuCl₂ solution 70 mg%
- Browin fixator (30 ml supersaturated solution of picric acid, 10 ml HCOOH and 2 ml CH₃COOH glaciar)
- Echinacea extract [4, 5,]

The alcoholic Echinacea extract (1:1 = C₂H₅OH:H₂O) standardized to 1% immunostimulatory polysaccharides, have been obtained from UMF Cluj and was subjected to a distillation process at 70-80°C degrees in order to remove the alcohol and to obtain a aqueous solution of Echinacea, that has been brought to the initial quantity of H₂O distilled [6,7].

**The method used**

In order to accomplish the experiment there have been used 7 sets of fertilized chick eggs, each of 60 eggs. Each set has been put into an incubator for 7 days at a temperature of 36-38°C degrees. After 34-38 hours in the incubator the eggs have been windows made, the embryos have been colored and observed with binocular glass, in order
to establish their age (the number of pairs of somites) and then they have been injected in
the caudal area under embryo with CuCl₂ solution and/or with watery solution of Echinacea.

The first sets have been injected with 50µl CuCl₂ 70mg%, the following two sets
with 25µl CuCl₂ 70mg%, another set with 25µl aqueous solution of Echinacea 1%
immunostimulatory polysaccharides and the last two sets with 25µl CuCl₂ 70mg% and 25µl
aqueous solution of Echinacea.

**Windows make**

From natural been wax, melted there have been modulated thin sticks for windows
make. We take the eggs from the incubator and put them on a support in the same position
as they were in the incubator. With the help of a dental drill we make an orifice at the
pointed pole of the egg and with a syringe we extract 3-4ml of albumen after that we close
up the orifice with wax. We cut rectangular pieces of plaster with which we cover the eggs
afterwards we cut up with the scissors a small window from the shell that was covered with
plaster of 2-3 cm² so that we have access to the embryo.

The wax stick is heated and stuck around the small window made in the egg under
the shape of a ring. After the embryo has been colored and observed with a binocular glass
and injected we apply over this wax ring windows make mount heated after we will seal the
whole in the shell. The operations must be displayed very efficiently so that egg stay few
time outside the incubator.

**The coloring** has been realized with a red neutral solution 0.2% that has been
applied over the surface of the embryo to has as result the contrast. Then the eggs have been
kept for 30 minutes in the incubator to accomplish the process of coloring.

**The observations under the binocular glass**

Each egg, at its turn is put on a support under the binocular glass. The egg is
pointed in such a way that the embryo can be observed in the visual field with the caudal
side towards the observer. The age and the phase of development of the embryo are
established through the counting of the somites pairs settled around the notochord. Each egg
will have a number and that will we written down between the number of somites pairs.
The injection is realized with the help of glass needles made from small tubes of glass through efilation at the flame. The process is achieved under the binocular glass in the caudal area under embryo. After all these procedures the eggs have been kept in the incubator and observed under the binocular glass daily for 7 days. In these days the embryo have been fixed and photographed.

The fixation is achieved with the help of a Bowin solution and for that it is needed that the embryos should be taken out from the egg and cleaned of the embryo’s membrane.

Taking photos have been made both in ovo and outside in the visual field of the binocular glass with the help of digital photo device.

The dates obtained from the observations made on all 7 sets of eggs have been presented and interpreted under a graphic shape. The graphics have been realized with the help of Microsoft Excel Program.

**Results and Discussion**

Notes used in this section: alive embryos without malformations (AnM); alive embryos with malformations (AM); dead embryos without malformations (DnM); dead embryos with malformations (DM).

The 7 sets eggs taken into consideration have contained 60 eggs each. There were kept 10 witnesses from each and the rest of embryos have been injected caudal under embryo area at the age of 34-38 hours of incubation. The results will be presented taken into consideration the treatment applied to the embryos in each set.

**Sets I and II**: injected with 50µl CuCl₂ 70mg%.

To the embryos injected we have been observed different changes such as: caudal area affected often, hemoragic point in the same area, caudal area unsettle, absent of caudal area, neural tube open and even death rate.

<table>
<thead>
<tr>
<th>Age</th>
<th>Witnesses</th>
<th>A.nM.</th>
<th>A.M.</th>
<th>D.nM.</th>
<th>D.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;13 somites</td>
<td>12</td>
<td>9</td>
<td>8</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>13-19 somites</td>
<td>7</td>
<td>10</td>
<td>4</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>&lt;19 somites</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>20</td>
<td>12</td>
<td>42</td>
<td>26</td>
</tr>
</tbody>
</table>
The graphs on the Figure 1 and 2 shows a high death rate (56%) among the chick embryos, as well as a teratogenic effect of CuCl₂ solution 70 mg%.

**Sets III and IV**: injected with 25µl CuCl₂ 70mg%.

<table>
<thead>
<tr>
<th>Table II. Results of set III and IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>&gt;13 somites</td>
</tr>
<tr>
<td>13-19 somites</td>
</tr>
<tr>
<td>&lt;19 somites</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
In this case of set III and IV, we observed the same changes, even if the doses were reduced.

**Set V**: injected with 25µl aqueous solution of Echinacea 1% immunostimulatory polysaccharides.

<table>
<thead>
<tr>
<th>Table III. Results of set V</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>&gt;13 somites</td>
</tr>
<tr>
<td>13-19 somites</td>
</tr>
<tr>
<td>&lt;19 somites</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
Observing the graphics on the figure 5 and 6 we can say that the watery solution of Echinacea 1% immunostimulatory polysaccharides don’t have negative effects on the development of the chick embryos.

**Set VI and VII:** injected with 25µl CuCl₂ 70mg% and then with 25µl aqueous solution of Echinacea 1% immunostimulatory polysaccharides.

### Table IV. Results of set VI and VII

<table>
<thead>
<tr>
<th>Age</th>
<th>Witnesses</th>
<th>A.NM.</th>
<th>A.M.</th>
<th>D.NM.</th>
<th>D.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;13 somites</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>13-19 somites</td>
<td>14</td>
<td>16</td>
<td>6</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>&lt;19 somites</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>22</td>
<td>3</td>
<td>28</td>
<td>44</td>
</tr>
</tbody>
</table>
These graphs, Figure 7 and 8, show us that administration of the aqueous solution of Echinacea 1% immunostimulatory polysaccharides immediately after injection with CuCl₂ 70mg% solution do not lead to a decrease of copper effects on chick embryos.

In the end we present some of the photos we have been taken, out of egg, that present different type of changes in caudal area.
Figure 9. In the left site is present an embryo that has a caudal area insufficient developed and in the right site is shown a normal developed embryo.

Figure 10. Embryo whit severe problems in caudal area

**Conclusion**

CuCl$_2$ 70 mg% solution injected in caudal area under embryo after 34-38 hours of incubation showed teratogenic and embryotoxic effects on the development of the chick embryo.

Aqueous solution of Echinacea 1% immunostimulatory polysaccharides injected also in caudal area under embryo after 34-38 hours of incubation don’t showed us negative effects on the development of the chick embryos.
The injection of the Echinacea solution immediately after injection of CuCl_2 solution don’t show us a decrease of the effects cause by copper on the embryos (the antioxidant effect remain disputable).

References

1. B. Barrett, “Medicinal properties of Echinacea”; Department of Family Medicine, University of Wisconsin Medical School, Madison WI, USA, 2003.