Study on Teratogenic Effects of Bhavana Panjankula Thailam, using Chick Embryo as a Model System

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ABSTRACT
Teratogenicity is the ability to cause developmental anomalies in fetus. Substances with teratogenicity effects can damage the DNA of a developing foetus. They may cause abnormal development of a limb malformation of an organ and the effects for the developing fetus can vary depending on the teratogen. Chicken eggs are used as a model system to test Bhavana panjankula thailam for its Teratogenic effect. Bhavana panjankula thailam is one of the Siddha drugs indicated for karuppachudu (a type of pitha disorder affecting uterus), urinary tract infections, dry skin and constipation. In this study five concentrations of drug Bhavana panjankula thailam were injected into the chicken embryos. Cadmium is used as a positive control in the same concentrations which affects limb deformities of the organ. 1:1 ratio of cadmium and drug were injected into the embryo for scrutinize the effect of drug. At the 12th day of incubation the eggs were dissected out and detect the malformation in the embryo. The results suggested that the lowest concentrations of drug treated embryo has no malformation and highest concentrations of drug treated embryo has malformed like heart and other organs are distorted. The DNA was isolated from embryos of chick and run in agarose gel electrophoresis to confirm the DNA damage. Coct4 and cGATA4 genes which is responsible for chick embryogenesis. GATA and OCT family of transcriptional regulatory proteins, GATA-4 and OCT-4 thought to be involved in the regulation of cardio genesis and gut development. Functions for these factors are known in the heart, but relatively little is implicit concerning their possible roles in the regulation of gut-specific gene expression. In this study, we analyzed the expression of cGATA-4, and cOCT4. Further gene expression studies will be carried out for further authentication of teratogenic effect at gene level. Though the Bhavana panjankula thailam was shown teratogenic effect at the higher dose So detailed animal studies and clinical trials should be carried out for apposite remedy.

Substances with known teratogenicity must be handled carefully. Pregnant women are encouraged to avoid exposure to such substances. As researchers have learned, however, sometimes the danger of a substance is not known until it is too late. Thalidomide, for example, was widely used in pregnant women until medical experts realized that it was causing developmental abnormalities.

Siddha System

The Siddha system is a treasure-house of secret science, embodying the results of the ordent pursuit there of by the ancient Siddhars. The contribution of Siddhar to Siddha literature with its boundless therapeutics and wonderful pharmaceutical preparation of medicine is acclaimed par excellence (pre-eminent) even in this 20th century and worthy of its remarkable results.

Siddha medicine revitalizes and rejuvenates dysfunctional organs that cause the disease and to maintain the ratio of Vaadham, Pitham and Kabham. The equilibrium of humours is considered as health and its disturbance or imbalance leads to a diseased state. So proper diet, medicine and a disciplined regimen of life are advised for a healthy living and to restore equilibrium of humors in diseased condition.

The Siddha treatment deals not only as a curative but also as a preservative, taking care of the external body with its internal being - soul. We don’t know about siddha drug fully for the any side effects, so analyse the siddha medicine to confirm the safety. Teratogenicity is the one assay to identify the drugs which
have any defects to fetus in pregnancy period of humans, so there is a need for an hour to perform teratogenicity study of the drug which will be more efficient to our society.

**Bhavana panjankula thailam**

Bhavana panjankula thailam is one of the siddha drug indicated for karuppachudu (a type of pitha disorder affecting uterus) urinary tract infection, drug skin and constipation. Bhavana panjankula thailam give to pregnant women. Bhavana panjankula thailam (SKM siddha and ayurvedha) is one of the Siddha drugs indicated for karuppachudu (a type of pitha disorder affecting uterus), urinary tract infections, drug skin and constipation. Bhavana panjankula thailam given to pregnant women.

**Dosage and Adjuvant**

2 to 5ml with milk or ghee twice a day after food or directed by the physician.

**Indication**

Ganachudu (A type of Pitha disorder), Karuppuachudu (A type of Pitha disorder affecting Uterus), Sirunee-pathai (Urinary tract infections), Sarira Varatchi (Dry Skin), Marunthukalin Ushnam (Body heat due to drugs), Malachikkal (Constipation). Advised for Pregnant ladies from the first month to the ninth month completion till delivery with dose of 5 ml. in the early morning empty stomach and 2.5 ml. at bed time by mixing with sugar candy which results in normal delivery and Child with good Physical and mental health.

**Materials and Methods**

**Study of Teratogenic activity**

**Materials for teratogenic activity on fertile chick egg**

**Drug**

- 1. Scissor
- 2. Forceps
- 3. Needle
- 4. Seal Tape
- 5. Dissection blade.

**Equipments**

1. Laminar flow hood
2. Freezer
3. Incubator
4. Shake
5. Centrifuge

**Reagents and Chemicals**

1. PBS
2. Cadmium
3. Ethanol
4. distilled water

**Glasswares**

1. Conical flask
2. Beaker
3. Test tubes
4. Beaker (250 ml)

**Dissection Tools**

1. Scissor
2. Forceps
3. Needle
4. Seal Tape
5. Dissection blade.

**Method for Teratogenic activity**

**Collection of Eggs**

Brown leghorn eggs were collected from government poultry station, potheri, Chennai. They were wiped with ethanol and kept for overnight incubation at temperature of 37 °C in an egg incubator.

**Treatment with Drug**

1. Zeroth day Fertile white leghorn chicken eggs were obtained from poultry and wiped with ethanol and was stored at 6°C.
2. These eggs were separated into 4 groups
3. Group-I as control untreated egg
4. Group-2 as positive control induced with cadmium
5. Group-3 treated with Bhavana panjankula thailam in various concentrations (0.5µg/ml, 50 µg/ml, 250 µg/ml and 500 µg/ml)
6. Group-4 treated with 1:1 ratio of Bhavana panjankula thailam and Cadmium
7. The eggs were then transferred and maintained in a forced draft egg incubator at 37.5°C with relative humidity of 46%.
8. After incubation period, eggs were sacrificed and observed for its malformations. Photographs were taken.

**Isolation of DNA**

**Materials for isolation of DNA**

**Equipments**

1. Laminar flow hood
2. Freezer
3. Incubator
4. Shake
5. Centrifuge

**Reagents and chemicals:**

1. PBS
2. Sodium Chloride
3. Potassium Chloride
4. Disodium Hydrogen Phosphate
5. Potassium Dihydrogen Phosphate
6. Phenol
7. Chloroform
8. SDS
9. Proteinase K
10. DNA Digestion Buffer

**Glasswares**

1. Conical flask
2. Beaker

**Method for isolation of DNA**

**Isolation of tissue from Chick Embryo**

1g of tissue was collected from the limb part of the chick embryo. It was then homogenized using Pestle and Mortar. The homogenized tissue was collected and stored in PBS.

**Isolation of DNA from the tissue sample**

**Procedure:**

**Quantification of DNA**

1. 0.1g tissue (grinds well using homogenizer/mortar and pestle/frozen with liquid nitrogen)
2. Add 0.5ml of DNA Digestion buffer with Proteinase K (can be stored at 4°C for several days)
3. Incubate overnight at 50 C-55 C with gentle shaking (if overnight cannot be done 1-2 hrs)
   Incubation/overnight for best results
4. Spin tubes for 5 seconds at 500 rpm to collect mix in bottom of tube.
5. Add 0.7ml of P: C: I (phenol: chloroform: is amyl alcohol)
6. Add 0.5ml of DNA Digestion buffer with Proteinase K (can be stored at 4°C for several days)
7. Incubate overnight at 50 C-55 C with gentle shaking (if overnight cannot be done 1-2 hrs)
   Incubation/overnight for best results
8. Add 1ml of DNA Digestion buffer with Proteinase K (can be stored at 4°C for several days)
9. Spin tubes for 5 seconds at 500 rpm to collect mix in bottom of tube.
10. Add 1ml of 100% ethanol and invert several times.
11. Centrifuge at 1000 rpm for 5 mins and discard supernatant
12. Air dry at room temperature for 10-15 mins
13. Re-suspend in 100µl TE buffer and incubate at 65°C for 15 mins to dissolve DNA.

**Materials Required**

**Equipments**
- Nanodrop 2000 – ThermoScientific

**Materials:**
- Micropipette – eppendroff
- Microtips - Tarsons
- Tissue paper

**Method of quantifying DNA**
DNA was quantified using Nanodrop 2000.

**AGAROSE GEL ELECTROPHORESIS**

**Materials Required**

**Equipments**
- Electrophoresis Power Pack - Genie
- Electrophoresis Gel tank - Genie

**Reagents and Chemicals**
1. Tris Borate EDTA (TBE Buffer) (1X) - Merk
2. Agarose (1.2%)
3. Bromophenol Blue
4. Ethidium Bromide

**Glasswares**
- Beaker
- Conical flask

**Method for Agarose gel Electrophoresis**

**Procedure**
1. Agarose prepared by 1X TBE buffer (1.2%) (0.48 g in 60ml d.h20).
2. 8µl of EtBr (for 60 ml) added in agarose.
3. Agarose was heated in oven and pour into mold, the comb was fixed.
4. After that solidification of agar the DNA sample was loaded (2ul tracking dye and 10 ul DNA)
5. Pour 0.5 X TBE buffer casted to wells
6. The electrode was set and 100 volts run the gel
7. After 2 hrs remove the gel and observe under UV illuminator for DNA damage.

**Polymearse Chain Resea**

**Materials required**

**Equipments**
- PCR- G-Storm
- Autoclave

**Chemicals and reagents**
1. Master Mix
   - PCR Water-Genei
   - PCR Buffer
   - Magnesium Chloride - Merk
   - dNTP - Invitrogen
   - Taq DNA Polymerase-Biolabs
2. Primers
   - cGATA4 – Sigma
   - cOCT4 – Sigma

**Materials**
1. PCR tubes- Sorenson
2. Microtips - Tarsons
3. Micropipette – eppendorff research

**Method for PCR**
1. Add 10µl of master mix
2. Add Forward primer 1µl
3. Add Reverse primer 1µl
4. Add DNA 3µl
5. Add PCR Water 5µl.

Three steps of PCR:
- Denaturation - 95°C for 2 minutes.
- Annealing - 55°C for 59 seconds
- Extension - 68-72°C for 45 seconds

PCR run for 40 cycles. Thus the DNA was amplified and agarose gel electrophoresis was carried out as above procedure.

**Result and Discussion**
Evaluate tertogenic effect using five concentration (50,100,250, 100 µg/ml(1:1) of BPT form the stock (10mg).cadmium used as positive control & untreated eggs used as negative vs control.

In this study 50 µg/ml concentration shows a good growth of embryo but in 250 µg/ml hear part oozing out and 50,100µg/ml concentration shows deformities like strunded growth was observed. The positive control of cadmium result in 250µg/ml concentration internal organ was damage and 500µ/ml concentration malformation where observed like limb deformities and one eye was not there in embryo the negative control untreated egg shows embryo was observed the normal there was no deformities.

**Results and Discussion**

**Results of teratogenic effect of Bhavana panjankula thailam**

This of isolated various concentration of DNA was run in Agarose gel electrophoresis (1:1) ladder used for easy to identity DNA withbase pair .In the control have normal DNA band and cadmium shows heavy DNA forgementation .In 50 µg/ml concentration of DNA mild DNA fregamation was seen but it better than 50µg /ml concentration ‘In100µg/ml(1:1) concentration shows verymild nucleotide feagementation was seen. So in this concentration drug was reading cadmium effect.
Composition of Bhavana panjankula thailam

<table>
<thead>
<tr>
<th>S.no</th>
<th>Siddha name</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amanakku vithai</td>
<td>Recinus communis</td>
</tr>
<tr>
<td>2</td>
<td>Chottrukattralai</td>
<td>Aloe barbadensis</td>
</tr>
<tr>
<td>3</td>
<td>Seuilaneer</td>
<td>Cocosnucifera</td>
</tr>
</tbody>
</table>

Table 1. Group 1 Negative control

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated egg</td>
<td>Fig 3</td>
<td>Embryo growth was normal and there was no deformities observed.</td>
</tr>
</tbody>
</table>

Table 2. Group 2 Cadmium treated eggs

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5µg/ml</td>
<td>Fig 4</td>
<td>Growth was normal and this indicates that at low concentration of cadmium, there was no malformations</td>
</tr>
<tr>
<td>50µg/ml</td>
<td>Fig 5</td>
<td>Embryo growth was normal and there was no malformations.</td>
</tr>
<tr>
<td>250µg/ml</td>
<td>Fig 6</td>
<td>Internal organs were damaged. Hence, malformations observed.</td>
</tr>
</tbody>
</table>
| 500µg/ml      | Fig 7       | Malformations were observed.  
(i)Limb Deformities  
(ii) One eye was not there in embryo. |
Table 3. Group 4 Bhavana panjankula thailam

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5µg/ml</td>
<td>Normal growth was observed.</td>
<td></td>
</tr>
<tr>
<td>50µg/ml</td>
<td>Normal growth was observed. There was no deformities.</td>
<td></td>
</tr>
<tr>
<td>250µg/ml</td>
<td>Malformation was observed. Heart part of the embryo came out.</td>
<td></td>
</tr>
<tr>
<td>500µg/ml</td>
<td>Deformities like stunted growth was observed.</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Group 5 Cadmium + Bhavana panjankula thailam

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>100µg/ml (1:1)</td>
<td>Deformities like stunted growth was observed.</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Interpretation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA Ladder (0.1-10 kbp)</td>
<td>This is marker which observe various size of DNA.</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>There is no DNA fragmentation observed in control which means (non induced) without treatment of drug.</td>
</tr>
<tr>
<td>3</td>
<td>Cadmium</td>
<td>DNA fragmentation and DNA damage is seen in cadmium teated band caused by cadmium.</td>
</tr>
<tr>
<td>4</td>
<td>Bhavana panjankula thailam (50 ug/ml)</td>
<td>Mild DNA fragmentation is observed with thick disorted band.</td>
</tr>
<tr>
<td>5</td>
<td>Bhavana panjankula thailam (250 ug/ml)</td>
<td>DNA fragmentation is seen. but damage is better than 50µg of treated one.</td>
</tr>
<tr>
<td>6</td>
<td>Bhavana panjankula thailam + cadmium (100ug/ml)</td>
<td>There is very mild DNA fragmentation is observed. because may be Bhavana panjankula thailam has reduced the DNA damage which caused by cadmium.</td>
</tr>
</tbody>
</table>

Table 8. Interpretation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>cGATA4 (Bhavana panjankula thailam 50ug/ml)</td>
<td>There is no traces of DNA.</td>
</tr>
<tr>
<td>2</td>
<td>cGATA4 (Bhavana panjankula thailam 250ug/ml)</td>
<td>There is no traces of DNA.</td>
</tr>
<tr>
<td>3</td>
<td>cGATA4 (Bhavana panjankula thailam+cadmium 100ug/ml)</td>
<td>There is no traces of DNA observed Cadmium damages the DNA</td>
</tr>
<tr>
<td>4</td>
<td>DNA Ladder</td>
<td>This is marker which observed various size of DNA</td>
</tr>
<tr>
<td>5</td>
<td>cOCT4 (Bhavana panjankula thailam 50ug/ml)</td>
<td>There is no traces of DNA</td>
</tr>
<tr>
<td>6</td>
<td>cOCT4 (Bhavana panjankula thailam 250ug/ml)</td>
<td>No traces of DNA found</td>
</tr>
<tr>
<td>7</td>
<td>cOCT4 (Bhavana panjankula thailam+ cadmium 100ug/ml)</td>
<td>DNA was not found in this gene. Cadmium damages the DNA</td>
</tr>
</tbody>
</table>
Discussion

The teratogenic effect of BPT has shows in the table3&4 along with the chick embryo(9). No malformation was observed in 0.5, 50µg/ml concentration & 250,500,100(1:1)µg/ml concentration of drug have deformities .In 250µg/ml concentration heart part was oozing out and 500,100(1:1)µg/ml concentration have stunted growth .the teratogenic effect of postive control cadmium was observed at two concentration (250/500µg/ml). the result of molecular level observation where shows in table no7&8 there DNA fregamation or damage was observed in drug treated as well as cadmium treated chick embryo .The level of DNA Damage was more in cadmium and lowest DNA(10). Fregamation compare than the concentration future the mechanism of activity of the drug analysed by gene expersison study .the standaration of protpol of the and analysis in PCR was bening this may be the standaration future propective of the present study of get intesting result about of teratogenic effect ofBPT.

The further detail analysis of gene expresison as well as molecular mechanism study of drug will be evavluated as a future study.

References