Estimating Teratogenic Potential of Garbhchintamani Rasa in Wistar Albino Rats by Biochemical and Histological Changes

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ABSTRACT

Introduction: Garbhchintamani Rasa is a herbo mineral drug used in Ayurveda during pregnancy that protects the fetus and also the pregnant mother from various diseases. In the present scenario, safety profile of drugs used in different stages of human development is very much mandatory. Thus the present study was aimed to evaluate the teratogenic potential of Garbhchintamani Rasa in Wistar albino rats. Method: The confirmed pregnant rats were divided into 4 groups of six rats each and drugs were administered till delivery. The teratogenic potential of test formulation was evaluated based on biochemical and histopathological examination of important organs in the offspring. Results: The biochemical and histopathological examination revealed that the test drug administered at therapeutic dose significantly increased uric acid and that of five times of therapeutic dose caused significant increase in the serum urea, Serum creatinine and uric acid levels, where as significant decrease in the serum SGOT was observed in comparison to normal control. The histopathalogical examination revealed almost normal cytoarchitecture in the therapeutic group, whereas at higher dose there was hyperemia, increased number of mature follicles. In ZoO treated group Mild to moderate epithelial proliferation in uterus, hyper activities of ovary and Myocarditis in heart were observed. Conclusion: Based on the findings it can be conclude that the test drug administered at therapeutic dose is relatively safer but at higher dose there is a chance of producing teratogenic potential.

KEYWORDS

Garbhchintamani Rasa, Histopathology, Myocarditis, Teratogenicity.

Received: 02.12.2016 Accepted: 08.02.2017 DOI: 10.5530/jams.2017.2.6

Safe mother hood is not a new idea for our Ayurveda. Starting from Garbhadana (Implantation), Masunumasika Garbhini paricharya (Month wise regimen during pregnancy) till Prasava Paricharya (care during Labour), all are aiming at getting healthy baby. During the gestational period, various drugs are advocated to prevent possible complications of pregnancy.[3] The normal growth and development of the fetus can be adversely affected by number of factors such as infection, teratogens, complication, and psychosomatic stress. Medicines have been tried to reduce the complexity of pregnancy and these drugs may cause teratogenic effect on the fetus.[2] A teratogen is an agent that acts during embryonic or fetal development period and produce a permanent or altered structure and function. Teratogen may be drugs, environmental factors, physical factors, chemicals or viruses. If a pregnant woman is exposed to any kind of teratogen, it may give birth to abnormal child.[3,4] Number of drugs prescribed during antenatal care (ANC) to the mother may hamper the development of the fetus. Assessments of these drugs are mandatory on the basis of toxicity and side effects. Many drugs have shown their teratogenic effect during animal experimentation and are been strictly restricted to be prescribed during pregnancy.

Ayurveda itself has the concept of Masanumasika Garbhini Paricharya, where the drugs were prescribed that eradicate the disease of pregnant woman and increases the bala (Strength), Varna (Colour), Buddhi (Intellectual power) etc in a newborn. Among such drugs, Garbhchintamani is one, which protects the fetus and helps to prevent various diseases of pregnant woman. It is explained in Bhaishajya Ratnakalai as Garbhini roga nashanarthi (Disease free status of pregnancy). It is one among the Rasaushadihis (Herbo mineral preparation), which contains Hingula (Cinnabar), Jatiphalo (Common nut mug), Pippali (Indian long pepper), Maricha (Black pepper), Shunti (Ginger root), Tankana (Borax).[5] Garbhchintamani is a drug advised in ANC with an intension to have a Shreysa Praja. (Healthy progeny). Garbhchintamani Rasa as the name suggests that which does care of Garbha and eliminate the disease of pregnant lady. However there is no data available on the safety aspects of the Garbhchintamani Rasa. Thus the present study was aim to evaluate the teratogenic potential of Garbhchintamani Rasa in Wistar albino rats by estimating biochemical & histopathological parameters of the rat offspring.
MATERIALS AND METHODS

Drug Preparation
The test drug Garbhachintamani Rasa (GCR) was prepared according to Bishajeya Ratnavali.[9] The in gradients such as Cinnabar, Borax and Jatiphal, Pippali, Maricha, Shunti were procured from SDM Pharmacy Udupi and same was authenticated by SDM Centre for Research in Ayurveda & Allied Sciences Udupi. Drugs like Cinnabar and Borax were subjected to purification methods as per Rasa Taramgini.[7] The final product in the powder form was taken for animal experimentation.

Experimental animals
Female Wistar albino rats of 200±50g weight body weight were used in the experimental study. The animals were procured from animal house facility attached to Pharmacology laboratory at SDM Centre for Research in Ayurveda and Allied Sciences. The rats maintained at standard laboratory conditions with natural day and night cycles with ideal laboratory condition of 25±2°C temperature and 55% humidity. They were fed with normal rat pellet supplied by Sri Durga feeds, Bangalore and tap water given ad libitum. The study protocol was approved from Institutional Animal Ethics Committee (CPCSEA/SDMCRA/IAEC-KP-01/2012-13).

Dose selection
The dose of Garbhachintamani Rasa (GCR) for human use was 250mg.[7] The rat dose was calculated from adult human dose based on body surface area ratio by referring to the Paget and Barnes table 1964.[8] The calculated dose was found to be 22.5mg/kg body weight and considered as therapeutic dose (TED). The GCR was administered in two different dose range i.e. therapeutic (TED) and five times therapeutic dose 112.5 mg/kg (TED X5) respectively. The test drug was made suspension in 0.5% CMC and five times therapeutic dose 112.5 mg/kg (TED X5) respectively. The test drug was made suspension in lukewarm water with 0.5 % CMC and administered at a dose of 1ml/100g body weight with the help of oral catheter. Animals were dosed consecutively from the day of confirmation of pregnancy till delivery.

Study design
Female rats with regular estrous cycle were allowed to mating with male rat and the pregnancy was confirmed by examining the presence of sperm plug in the vaginal smear. Thus confirmed pregnant rats were categorized into 4 different groups of six rats each. Group I administered with suspension of 0.5% CMC in lukewarm water and maintained at normal rat diet and water considered vehicle control. Group II administered with proven teratogenic chemical Zink Oxide (ZnO) at a dose of 250mg /kg body weight and considered as toxicant control. Group III & IV were administered with GCR at therapeutic and five times of therapeutic dose with anupana. The group specific drugs were administered throughout the pregnancy from the day of pregnancy confirmation till the delivery. During the experimental period, general behavior and body weight changes were observed. On the final day the rats were anesthetized and blood was withdrawn from retro orbital plexus and biochemical investigations were carried out.[9] The organs such as anterior pituitary, heart, kidney, liver, uterus and ovary samples from each group were examined for histopathological study. Immediately after the excision from rats, the tissues were transferred into 10% formalin. Sections of 5 μm thickness were prepared using microtome and stained with haematoxyline and eosin for microscopic observations.[9] All slides were then evaluated under light microscope (ZEISS Axiolab A1 India).

Statistical analysis
The data obtained were presented as Mean ± SEM. The difference between or among the groups was analyzed by employing one way ANOVA followed by Dunnet’s multiple t-test as post hoc test. Graph pad Inst 3 was used for this purpose. A P value of less than 0.05 was considered to indicate statistically significant.

RESULTS AND DISCUSSION
Zinc oxide administered group has shown significant elevation in the liver enzymes SGOT, ALP and uric acid level in comparison to normal control. The test drug GCR administered at therapeutic dose level has shown significant increase in the blood uric acid level whereas the GCR administered at five times of its therapeutic dose has shown significant increase in the serum SGOT, blood urea, Serum creatinine and uric acid level in comparison to normal control (Table 1).

Table 1. Effect of Garbhachintamani Rasa on biochemical parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT IU/L</th>
<th>SGPT IU/L</th>
<th>ALP IU/L</th>
<th>Blood urea (g/dl)</th>
<th>Blood uric acid (mg/dl)</th>
<th>Sugar mg/dl</th>
<th>Total protein (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>139.33 ± 3.61</td>
<td>84.66 ± 7.07</td>
<td>449.5 ± 60.16</td>
<td>33.66 ± 2.04</td>
<td>0.58 ± 0.03</td>
<td>104.66 ± 4.51</td>
<td>46.66 ± 17.25</td>
<td>0.6±0.07</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>219.83 ± 9.68**</td>
<td>100.33 ± 11.31</td>
<td>742.85 ± 88.50**</td>
<td>34.33 ± 2.09</td>
<td>1.73 ± 0.04**</td>
<td>125.83 ± 6.89</td>
<td>53.33 ± 14.75</td>
<td>0.71±0.04</td>
</tr>
<tr>
<td>GCR-TED</td>
<td>129.83 ± 7.28</td>
<td>63.16 ± 3.68</td>
<td>399.83 ± 28.35</td>
<td>39.83 ± 3.41</td>
<td>1.8 ± 0.17***</td>
<td>104.66 ± 7.25</td>
<td>53.33 ± 14.75</td>
<td>0.67±0.06</td>
</tr>
<tr>
<td>GCR-TED X5</td>
<td>108.16 ± 7.02*</td>
<td>58.66 ± 5.41</td>
<td>515.66 ± 73.36</td>
<td>45 ± 3.17*</td>
<td>2.6 ± 0.24***</td>
<td>123.66 ± 4.66</td>
<td>46.66 ± 17.25</td>
<td>0.83±0.03**</td>
</tr>
</tbody>
</table>

Data expressed in Mean ± SEM, *P<0.05, **P<0.01 in comparison to control group. GCR- Garbhachintamani Rasa, TED- Therapeutic dose.

The histopathological examination of anterior pituitary, liver, heart, kidney, ovary and uterus revealed almost normal cytoarchitecture in GCR administered at therapeutic dose, where as GCR at 5 times of TED showed a hyperemia and increased number of mature follicles in ovary as compared to control group. In ZnO treated group Mild to moderate fatty changes in the liver, epithelial proliferation in uterus, hyper activities of ovary and Myocarditis in heart were observed (Figure 1-6).
Figure 1. Photomicrograph of Anterior pituitary

1.1 Control group, 1.3-1.4 Zink Oxide treated group, 1.5-1.6 test drug GCR (TED), 1.7-1.8 test drug GCR (TED X5)

Figure 2. Photomicrograph of heart tissue
Figure 3. Photomicrograph of kidney tissue
Figure 4. Photomicrograph of liver tissue

Figure 5. Photomicrograph of ovary tissue
Figure 6. Photomicrograph of uterus tissue
Blood Urea contributes most of the body non protein nitrogen, accounting for about 45% of the total requirement. It is major end product of protein catabolism synthesized in liver, released in blood circulation and excreted by the kidneys. It is a chief indicator of renal and hepatic integrity. Elevated serum urea level may be due to prerenal, postrenal and renal etiology. Pre renal causes could be cardiac related or increased protein catabolism. Renal causes including glomerulonephritis, chronic nephritis, nephritic syndrome or other kidney disease. Post renal causes include obstruction of urinary tract etc. Serum creatinine formation begins with the transamination from arginine to glycine form glycochymine or guanidoacetic acid (GAA). This reaction occurs primarily in the kidney. In all stages of insufficiency, the serum creatinine is a more reliable indicator of renal function than the blood urea because the blood urea is far more likely to be affected by dietary and physiologic conditions. High level of blood urea and Serum creatinine applies in moderate to advanced renal failure. The increase in serum urea and Serum creatinine in higher dose of Garbhachintamani is likely hood of kidney damage even when absolute values were still in normal range.\(^\text{[11]}\)

The uric acid is considered as end product of purine metabolism and excreted to a larger extent by the kidney and to a smaller degree in the intestine tract by microbial degeneration. In kidney uric acid is freely filtered by the glomeruli, partially excreted in the proximal convoluted tubules. Significant increase in the uric acid level is found in all three groups may be due to impairment in the purine metabolism and renal damage. Whereas in test drug administered at therapeutic dose having normal blood urea level and increased uric acid as compared to normal control. Here increased uric acid may be due to impairment in the purine metabolism or because of renal parenchyma damage.\(^\text{[12]}\)

SGOT is present in different tissues having its highest concentration in heart followed by liver, skeletal muscle, kidney, pancreas, spleen, lung and erythrocytes.\(^\text{[13-15]}\) Statistically increased SGOT value seen in ZnO and GCR (TEDx5) groups shows destructions of cell not only in liver but also seen in other organs.

Test drug GCR administered at therapeutic dose showed almost normal cytoarchitecture in anterior pituitary, heart, liver, kidney, uterus and ovary, whereas at five times of therapeutic dose showed mild to moderate hyperemia and increased mature follicles in ovary as compared to control group. The zinc oxide administered group has showed mild to moderate fatty changes in the liver and hyperactivity with increased follicles in the ovary as compared to the control group. Thus based on the biochemical and histopathological changes we could confirm in higher dose there is a chance to develop toxicity in the developing fetus however at the lower dose range it is relatively safer and showed almost normal biochemical and histopathological parameters.

**CONCLUSION**

Present study revealed the test drug Garbhachintamani Rasa administered at therapeutic dose showed relatively normal biochemical and histological changes, whereas at five times of therapeutic dose showed mild to moderate toxic changes by increasing the serum urea and creatinine level and also does hyper stimulation of organs observed in offspring and indicative of teratogenic potential. Thus we could conclude that the GCR administered at classically indicated dose is relatively safer; however there is a chance to develop toxic signs in the fetus at higher dose levels. Thus more emphasis is given on dose selection.
CONFICT OF INTEREST
Nil

REFERENCES

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GRAPHICAL ABSTRACT


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