ISSN 2456-3110
Vol 2 · Issue 1
Jan-Feb 2017

Journal of Ayurveda and Integrated Medical Sciences

www.jaims.in

JAIMS

Charaka Publications

Indexed

ISSN: 2456-3110 ORIGINAL ARTICLE Jan-Feb 2017

Teratogenic Potential of *Garbha Chintamani Rasa* in Wistar Albino Rats with focus on physical and behaviour changes

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ABSTRACT

Teratogens are the substances that may produce physical or functional defects in the human embryo or after the pregnant woman exposed to the substance. Exposure to the teratogenic drugs affects the foetus or embryo in a variety of ways, such as the duration of exposure, the amount of teratogenic substance, and the stage of development the embryo or foetus is in during the exposure. Teratogens may affect the embryo or foetus causing physical malformations, problems in the behavioural or emotional development of the child, and decreased intellectual quotient (IQ) in the child. The present study was carried out to assess the teratogenic potential of *Garbhachintamani Rasa* in *Wistar albino* rats based on physical and behaviroal abnormalities. The experiment was designed in such a way that the conformed pregnant rats were selected and administered with *Garbhachintamani Rasa* for 21 consecutive days. The delivered pups were assessed for teratogenicity based on the physical and behavioural parameter in a set of behaviroal tests such as open field test, rota rod and swimming test at different developing periods. The results showed that, there was significant physical and behavioural alteration in the test drug administered at higher dose level as compared to normal control. Thus it can be concluded that the test drug GCR at therapeutic dose showed well tolerated and nearly normal behavioral pattern, whereas at higher dose it can cause behavioral abnormalities in pups.

Key words: *Garbhachintamani Rasa,* Teratogenicity, Open field test, Rota rod test.

INTRODUCTION

Pregnant women health has become the prime concern in present time and her fitness in physical as well as mental has become chief objective of Obstetrician. The normal growth and development of the foetus can be adversely affected by number of factors such as infection, teratogens, complication and psychosomatic stress. Medicine have been tried to reduce the complication of pregnancy and these drugs may cause teratogenic effect on the foetus.[1],[2]

Teratogens are substances that may produce physical or functional defects in the human embryo or after the pregnant woman is exposed to the substance. The Teratogens may be chemical agents may be drug, physical, diet, genetic influence and viruses etc. Sensitivity to teratogen induced malformation varies during different developmental stages at the time of exposure, where there are critical periods of sensitivity to agents and organ systems. Teratogens act via a specific mechanism on developing cells and tissues to initiate a cascade of altered developmental events. Teratogenic effects are dependent on the

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Submission Date : 05/02/2017 Accepted Date: 30/02/2017

Access this article online

Quick Response Code

Website: www.jaims.in

DOI: 10.21760/jaims.v2i1.7479

ISSN: 2456-3110 ORIGINAL ARTICLE Jan-Feb 2017

nature of the teratogen, including chemical properties of the chemical, route of exposure, maternal / foetal bio activation, placental transport, etc. Teratogens produce a consistent deviation from normal development. Deviation can include: (1) death, (2) malformation, (3) growth retardation, or (4) functional defect. Teratogen induced malformations occur in a dose dependent manner, ranging from no observable defects to total lethality.[3],[4]

Ayurveda itself has the concept of *Masanumasika Garbhini Paricharya* (Month wise regimen during pregnancy), where the drugs are prescribed to eradicate the disease of pregnant woman and increases the *Bala (strength), Varna* (Complexion), *Buddhi* (Intellectual power) etc. characteristic in newborn. Among various formulation *Garbhachintamani* is one which has been advised in ANC with an intension to have a *Shreyasi Praja*. *Garbhachintamani Rasa* as the name suggests means that which does care of *Garbha* and which helps the *Garbha* in its development. It is one among the *Rasaushadhis* which contains *Hingula (cinnabar), Jatiphala (Indian nut mug), Shunti (Ginger root), Pippali (Piper longum), Mareecha (Black Pepper), Tankana (Borax)* with *Ushnodaka* as *Anupana*.[5],[6]

All the *Bhasmas* after being tested for their authenticity are mixed with the *Kastaaushadhis* (Herbal drug) and *Bhavana* of *Jambeera Swarasa (Lemon juice)* and then small pills of *1 Ratti Pramana* is prepared. It is given to the pregnant lady in 2 Ratti *Pramana* daily till the day of delivery. It helps in *Garbhini Roga Nashanarta* (Disease free status in pregnancy).[7] Number of drugs were prescribed during ANC to the mother may hamper the development of the foetus. Assessment of these drugs is mandatory on the basis of toxicity and side effects. So it is need of a time to assess teratogenicity of *Garbhachintamani Rasa* in pregnant albino rats with focus on Physical and behaviour changes.

Materials and Methods

Experimental animals

Female Wistar albino rats of weighing between 200±50g body weights were used for 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 condition with natural day and night cycles with ideal laboratory condition of 25±2 ^{0} c temperature and 55% humidity. They were fed with normal rat pellet supplied by *Sri Durga* feeds from Bangalore and tap water given *ad libitum.* The study protocol was approved from Institutional ethical committee with IAEC no. SDMCRA/IAEC-2012-13 and principles of laboratory animal care guidelines were followed throughout the experimentation.

Test drug preparation

The test drug *Garbhachintamani Rasa* (GCR) was prepared according to the Ayurvedic formulary of India. Raw drugs and Cinnabar was authenticated in the pharmacy. Authentication was done by evaluating their quality by various parameters. Identification of herbs and their Pharmacognosy characters were done at SDM research for allied science. All the pharmaceutical procedure like *Shodhana* (purification procedures) were carried out in the *Rasashastra* practical hall SDMCA.

Dose selection

The dose of *Garbhachintamani Rasa* (GCR) for human use is 2 *Ratti* (125mg per ratti) according to classical text of *Rasashastra.*[8] The rat dose was calculated from adult human dose based on body surface area ratio by referring to the Paget and Barn's table 1964.[9] 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 x 5) respectively. The test drug was made suspension in 0.5% CMC and administered at a dose of 1ml/100g body weight with the help of oral catheter.

Study design

The selected animals were divided into four groups, with each group comprising of six pregnant rats. The first group kept under control, second group

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considered as toxicant control and administered with Zink oxide. The 3rd and 4th groups were administered with test drug *Garbhachintamani Rasa* (GCR) at therapeutic and five times of therapeutic dose. After delivery, six pups has been taken from each group, physical development and behavioural changes like motor skill- swimming development, negatives geotaxis test, rota rod test, reflex development- surface righting reflex, cognitive functions - ascending wire mesh test, behavioural function - open field exploration test and weight gain were assessed in the prenatal and postnatal period.

Statistical analysis

The data obtained were presented as Mean ± SEM. The difference between or among the groups was analysed 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

The *Garbha Chintamani Rasa* (GCR) administered at therapeutic dose has shown significant decrease in the body weight measured on 15th day of gestational period in comparison to normal control, whereas other groups has shown normal growth pattern and comparable with that of normal control. (Table 1)

Table 1: Effect of test drug on pregnant rat's body weight measured at different gestational period.

Group	Gestational period (days)						
	3rd	6th	9th	12th	15th	18th	21st
Normal Control	195±10.28	205±9.02	228.16±9.39	240.16±11.32	252±9.81	269.66±12.59	282.83±9.87
Zink Oxide	187.5±4.08	213.83±9.26	224.16±9.05	233.83±9.89	247±5.63	261.83±8.22	218±40.24
GCR (TED)	210.66±8.89	224.33±3.16	233.5±4.75	239.66±3.66	209.16±6.24**	226.5±8.04*	247.16±10.16
GCR (TEDx5)	209±5.13	192.16±4.54	203.83±6.11	208±6.10	248.66±11.53	259.83±9.48	279±12.03

Data: MEAN ± SEM, *P<0.05, **P<0.01 in comparison to normal control, GCR- *Garbha Chintamani Rasa, TED* - Therapeutic dose, TED x 5 - Five times of therapeutic dose.

There is a significant decrease in the neonatal birth weight in Zinc oxide administered group and GCR at five times of therapeutic dose as compared to the normal control, whereas the GCR at therapeutic dose has shown normal pups weight and comparable with that of normal control. (Table 2)

Table 2: Effect of test drug on the birth weight of the neonates.

Group	Pup's Weight (g)
Control	6.44±0.17
Zink Oxide	5.45±0.15**
GCR(TED)	6.5±0.18
GCR(TEDx5)	5.53±0.15**

Data: MEAN ± SEM, **P<0.01 in comparison to normal control, GCR- *Garbha Chintamani Rasa,* TED- Therapeutic dose, TED x 5 - Five times of therapeutic dose.

There was significant increase in the body weight of off spring administered with therapeutic dose of GCR on 15th and 18th days after birth whereas GCR administered at higher dose has shown nearly normal body weight comparable with that of control group. The zinc oxide administered pups has shown significant decrease in the body weight measured on 15th, 18th and 21st days after birth as comparable with that of control group. (Table 3)

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Table 3: Effect of test drug on the birth weight of the offspring's at different growing period.

Group	Growing period (days)						
	3rd	6th	9th	12th	15th	18th	21st
Normal Control	7.5±0.22	9±0.00	13.66±0.33	15.66±0.33	19±0.25	23.16±0.40	26.5±0.67
Zink Oxide	6.33±0.33	9.56±0.81	11.5±0.632	13.5±1.45	15.33±0.66**	18.66±0.55**	24.66±1.08
GCR (TED)	8.69±0.48	11.5±1.23	15.66±1.38	19.05±0.28	22.16±0.70*	28.83±0.54**	32.83±1.13**
GCR (TED x5	6.66±0.21	9±0.36	13.5±0.61	16.33±0.33	20±1.03	22.5±0.84	27.66±0.61

Data: MEAN ± SEM,*P<0.05, **P<0.01 in comparison to normal control, GCR- *Garbha Chintamani Rasa,* TED - Therapeutic dose, TED x 5- Five times of therapeutic dose.

There is a significant increase in the surface righting reflex measured on 3rd and 5th day after birth in Zinc oxide administered group as compared to normal control. The GCR administered at both dose levels has shown considerably higher righting reflex time and comparable with that of normal control. (Table 4)

Table 4: Effect of test drug on Achievement of surface righting reflex after 3rd 4th & 5th day after birth.

Group	Duration (sec)		
	3rd day	4th day	5th day
Normal Control	3.33 ± 0.42	2.33 ± 0.21	2.16 ± 0.16
Zinc oxide	24.83±11.13*	4.16±0.79	4.00±0.51*
GCR (TED)	4.16 ± 0.16	2.66 ± 0.33	1.66 ± 0.21
GCR (TED x 5)	8.00 ± 1.91	4.83 ± 1.16	2.00 ± 0.21

Data: MEAN ± SEM,*P<0.05 in comparison to normal control, GCR- *Garbha Chintamani Rasa, TED*- Therapeutic dose, TED x 5- Five times of therapeutic dose.

There is a significant decrease in the angle development measured on 8th, 10th and 12th day after birth in Zinc oxide administered group as compared to normal control. The GCR administered at higher dose level has shown significant decrease in the angle development on 8th day whereas on 10th and 12th day both dose levels has shown nearly normal angle development, comparable with that of control group. (Table 5)

Table 5: Effect of test drug on Achievement of angle development measured after 8th, 10th 12thday's of birth.

Group	Duration (sec)		
	8th day	10th day	12th day
Normal Control	4.66 ± 0.21	4.83±0.16	5.00 ± 0.00
Zinc oxide	2.83±0.16**	4.16±0.16*	4.16±0.16**
GCR(TED)	4.66 ± 0.21	4.83±0.16	5.00 ± 0.00
GCR(TEDx5)	3.66 ± 0.33*	4.33 ± 0.21	4.66 ± 0.21

Data: MEAN ± SEM, *P<0.05, **P<0.01 in comparison to normal control, GCR- *Garbha Chintamani Rasa, TED*- Therapeutic dose, TED X5- Five times of therapeutic dose.

There is a significant decrease in the score given for limb movement on 8th and 10th day after birth in GCR administered at higher dose and zinc oxide on 10th day as compared to the normal control, whereas the GCR administered at therapeutic dose has shown non - significant and nearly normal value comparable with that of control group. (Table 6)

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Table 6: Effect of test drug on Achievement of Limb movement in swimming test measured after 8th, 10th 12th days of birth.

Group	8th day	10th day	12th day
Normal Control	2.83 ± 0.16	3.00 ± 0.00	3.00 ± 0.00
Zinc oxide	2.16±0.16	2.16±0.16**	2.16±0.16
GCR (TED)	2.66 ± 0.33	3.33 ± 0.33	3.00 ± 0.00
GCR (TEDx5)	1.66±0.21**	1.83±0.16**	1.83±0.16

Data: MEAN ± SEM, **P<0.01 in comparison to normal control, GCR- *Garbha Chintamani Rasa, TED*- Therapeutic dose, TED X5- Five times of therapeutic dose.

The GCR administered at higher dose has shown significant increase in the time taken on 10th day after birth in the negative geotaxis test whereas GCR at therapeutic dose has shown nearly normal as compared to normal control. The zinc oxide has shown significant increase in the time taken in the negative geotaxis test on 10th and 12th as compared to normal control. (Table 7)

There was no significant difference in the time taken for ascending wire mesh test in test drug administered group as compared to normal control. (Table 8)

The Zinc oxide administered group rats have shown significant decrease in the peripheral and central crossing and rearing behaviour in the open field behaviour test as compared to the normal control. The test drug administered at higher dose level has shown significant decrease in the peripheral and central crossing as compared to the normal control group. (Table 9)

In the rota rod test was conducted on 22nd and 49th day after birth for all the group rats and found no difference between the groups and comparable with that of normal control. The Zinc oxide and GCR (TED x 5) administered group rats have shown significant decrease in the retention time as compared to the normal control. (Table 10)

DISCUSSION

Deletion and prevention of foetal abnormalities has become the major aspect in the antenatal care. Parents are intending to have a small family of a single child where they always try and hope to have a healthy and good qualities. Hence it becomes the responsibility of the obstetrician to be very careful in prescribing medicine to the pregnant mother. Many drugs have shown their teratogenic effect during animal experimentation and are been strictly restricted during pregnancy. *Garbha Chintamani Rasa* is a drug which is been supplemented to the mother as it cures the diseases and protect the baby. With an idea to investigate the possible effect of *Garbhachitamani Rasa* on the offspring the present study was under taken i.e. to see the efficacy, side effects if any of *Garbhachintamani Rasa.*

In the present study there was significant decrease in the pup's birth weight in zinc oxide and GCR administered at higher dose level as compared to the normal control. This indicates there is an influence of GCR on the normal physiological growth of pups.

The open field behaviroal test is an experimental procedure used to assay general locomotors activity as well as anxiety behaviour in rodents. In the present study the zinc oxide and GCR at higher dose level showed significant decrease in the peripheral and central crossing and considerable decrease in the rearing behaviour. This indicates that both the drugs has a potential to cause CNS depression property.[10],[11]

The surface righting reflex is a combination of visual system inputs, vestibular inputs, and somatosensory inputs to make postural adjustments when the body becomes displaced from its normal vertical position. These inputs are used to create an efference copy. This means that the brain makes comparisons in the cerebellum between expected posture and perceived posture, and corrects for the difference. The reflex can be affected by various types of balance

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disorders.[12],[13] In the present study there is a significant decrease in the time taken by zinc oxide administered pups for showing normal righting reflex during 3rd day after birth, where as 5th day same pups they showed significant increase as compared to normal control. The observed changes might be due to interference of zinc oxide central nervous system function especially with that of cerebellum.[3] GCR administered at higher dose level significantly prolonged the time taken in the negative geotaxis behaviroal test conducted on 12th day after birth as compared to normal control. This indicates GCR at higher dose has a potential to hamper normal physiological growth.

There was significant decrease in the retention time in the rota rod test in zinc oxide administered group as well as GCR at higher dose level in comparison to normal control. The rota rod test is mainly used to assess the muscle strength and coordination in rodents. The drugs with skeletal muscle relaxant property or CNS depression property will increase the retention time at a particular rpm. The rota rod test was performed 22nd and 49th day after birth. The GCR at higher dose has shown significant reduction in the retention time on 49th day after birth and gives an evidence of potential to cause CNS depression or muscle relaxant property.

CONCLUSION

It can be concluded that the test drug at therapeutic dose level showed no toxic potential where as at higher dose level it produced marked CNS depression as well as hampered normal physiological growth of pups. Thus GCR is relatively safer at therapeutic dose.

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How to cite this article: Prathviraj Puranik, VNK Usha, B Ravishankar, Mundugaru Ravi. Teratogenic Potential of Garbha Chintamani Rasa in Wistar Albino Rats with focus on physical and behaviour changes. J Ayurveda Integr Med Sci 2017;1:1-6.
http://dx.doi.org/10.21760/jaims.v2i1.7479

Source of Support: Nil, **Conflict of Interest:** None declared.