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TERATOGENIC EFFECT OF SWASA-KASA-CHINTAMANI-RASA (SKCR) IN ANIMAL MODEL

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ABSTRACT

The drug Swasa-Kasa Chintamani Rasa, a herbometallic drug is commonly used for the treatment of bronchial asthma in the children. This study was designed to evaluate its teratogenic effect, if any, on the fetuses of pregnant mice. The healthy mice of 120 days old 10 male and 10 female of Swiss strain were selected with an average weight 25 grams. The drug in a dose of 16mg/kg/day was given orally from 5th day to 9th day of gestation once in a day for 5 consecutive days to mice of group-A, while the mice in Group 'B' were given only distill water of same dose. On Day 18thFetuses were collected after scarifying the pregnant mice. Mean weight and length of fetuses of group A were observed significantly reduced than group B fetuses. Histological findings of treated groups have shown reduction in neuronal population, edema, scanty Kuffer cells, degeneration and apoptosis etc. in the tissues. Hence these findings suggest that Swasa-Kasa-Chintamani-Rasa [SKCR] produces embryo toxic effect on the developing mice.

KEYWORDS: SKCR, embryotoxic.

INTRODUCTION

Teratology is the study of abnormal development in embryos and the search of possible cause of congenital malformation or birth defects (Wendy Chung, 2004).[1]

The WHO survey indicated that about 70-80% of world populations rely on non-conventional medicine mainly of herbal source in their primary health care (Chan K., 2003).[2] Many *Ayurvedic* drugs had also been proved to be teratogenic like methanolic extract of Asparagus

racemosus (Shatavari) [Singh G. et al, 2005].[3] extract of Solanum xanthocarpum and Adhatoda vasica (Nath et al, 1992).[4]

About 8% of pregnant women need drug treatment due to their chronicity of diseases including bronchial asthma (Czeizel et al, 1999).[5] Drugs used during the pregnancy may pose a teratogenic effect to the embryo. Mercury exposure is the second most common cause of toxic metal poisoning in humans. (Lyn, 2002).[6]

In Ayurveda, SKCR (Shvas-Kasa-Chintamani-Rasa) is considered as the drug of choice for asthma (R.S.S. 3/19-21, Brihad RasrajaSundaram, Shvasadhikar Page.s No. 438, Rasa-tantrasar and Siddha Phrayoga Samgarh 2nd Vol. 13/1, B.R.Chi 16/57-60), which is prepared from the Kajjali, Svarna(Au), Lauha(Fe), Abhraka (Ash of biotite mica), Mukta Bhasama (pearl powder) and Swarna Bhasma (gold powder) triturated with Kantakari (Solanum surattense), Ardraka (Gingiber officinale), Aja-dugdha (goats milk), Mulethi (Glycrrhiza glabra) and Pana svarasa (extract of piper bettle) as per the process recommended in text (R.S.S. 3/19-21). On analysis of SKCR drug suggest presence of mercury, iron, sulphur and copper. In SKCR, mercury is present in form of mercuric sulphide (HgS), copper as copper nitrate (Cu (N03) 2) and Iron as Iron Hydroxide (Fe (OH) 3) in the different molecular form (Kumar Y et. al. 2008).[7]

Review of Literature

It is well known that inorganic mercury cross the placenta of animals [Dock L et al. 1994, Nordenhall K et al. 1995, and Mansour M. M. et al. 1973].[8] Inorganic mercury viz. methyl mercury has been detected in fetal liver and brain of hamster [Kajiwara, Y., and M. Inouye, 1986].[9] It induces embryotoxic effects [Gale T.F. et al 1974, 1981 & 1984].[10,11,12] Subcutaneous injection of mercuric acetate to dams produced a variety of malformation like cleft palate, hydrocephalus, and heart defects in mice [Gale T.F. et al, 1971].[10] Embryotoxic effect in pregnant mice and rats was also observed after getting exposure to mercuric chloride [Khan A.T. et al. 2004].[13] [Kavlock R et al. 1993].[14] Prenatal methyl mercury exposure can result in communicating hydrocephalus in 15% to 25% of surviving offspring and examination of serially sectioned cerebral aqueduct in hydrocephalic animals had revealed the presence of stenosis but not the complete occlusion of the lumen. The ependymal cells of the cerebral aqueduct showed no evidence of periaqueductal inflammation or reactive gliosis. Edema and vacuolar changes were, however, observed subependymally. The cerebral white

matter showed edema, spongy degeneration, gross cystic change and loss of parenchyma [Bartolome J., et al 1984].[15]

Aims and objective

The developing fetus has many fold probability for developing or having teratogenic effect when exposed to heavy metals like mercury. Many of the Ayurvedic physicians do not agree with this due to lack of scientific studies of mercurial Ayurvedic preparation on the fetuses and insignificant histopathological changes on the vitals organs of mice (Upadhayay et al. 2008).[16]

Therefore the teratogenic effect of SKCR in the animal model is essential for the irrational use of drug containing mercury.

MATERIALS AND METHODS

To assess the teratological effect, if any, of the SKCR drug, this study was designed in Dep‟t of Kaumarbhritya/ Balaroga, Faculty of Ayurveda and was carried out in Dep‟t of Anatomy, Faculty of Modern Medicine, IMS, BHU, by procuring 120 days old 10 female and 10 male Albino mice of Swiss strain from the animal house of IMS, BHU as per the inclusion and exclusion criteria.

Inclusion

Healthy mice, which were not used in any experimental study used as the control and treated groups.

Exclusion

For the present study, the following criteria were taken –

Any illness appear during the study period or suffering from disease at the starting point i.e. before mating.

All the twenty mice were divided into two groups „A‟ & „B‟. Each group has 5 male and 5 female mices. Female mice of Group„ A‟ were treated with SKCR while the mice of Group „B‟ received only distilled water from day 5th to 9th of gestation (table no.1).

Table no 1:

Group	M	F	Drug	Dose
A (n=10)	5	5	SKCR	16mg/kg/day (Two times of daily dose)
B (n=10)	5	5	Distill water	1ml

Mating

Female mice were kept overnight with the male partner of the same stock housed in same cages at 6.00pm in evening (1:1 ratio). Next morning at 6.00am mice were examined for vaginal plug to confirm presence of sperm positive. The fertilization, which was assumed to occur after observing the vaginal plug, was designated as day „0" of the gestation. Thereafter, the pregnant mice were kept in separate cage and were fed with „Hind lever diet" and mineral water ad libitum. The temperature was maintained around 25 ± 1ºC and relative humidity at 50% to 70%. Weight of each mice of each group were also taken on day 0 then day 3rd, day 9th and on day 18th to assess the change of weight during pregnancy.

Dose Calculation

The study was planned to access teratological effect of Drug SKCR on mice with dose starting as 2 times of TED, 5 times of TED, 10 times of TED, but as teratogenic effect was found even on 2 times of TED so further study was cancelled (CCRAS/OECD guidelines).

The dose of SKCR was calculated as: -

Average weight of female mice was 25 gram (25.4±1.94) and 16 mg/kg/day given to all the female mice of group „A"

Therefore 25gm= 16/1000 □ 25=0.4mg

Therefore, 0.4mg/day pre-calculated dose of SKCR was given as per schedule to each female albino mice.

Procedure for drug administration

10 mg drug was dissolved in 10 ml distilled water and was vigorously shacked to form homogeneous suspension (Photo 0). Each mice of Group A, 0.4 ml of drug suspension was given orally, from 5th day to 9th day of gestation, once in a day for 5 consecutive days.

For that purpose, the prepared drug was drawn into the sterilized syringe, which had curved and blunt needle. Calculated dose of the prepared drug was drawn and given by keeping the mice in supine position. (Photo 1)

After the drug administration on pregnant mice their motor activity and behavior pattern was observed on every half an hour, 1 hour, 2 hour, 4 hour and 24 hour. No significant change in motor activity and behavior pattern was observed in mice‟s of either group.

Collection and fixation of embryos

Control group mice as well as SKCR treated female mice were sacrificed after giving excess of ether anesthesia on day 18th of gestation. Then fetuses were collected by means of laparotomy.(Photo 2). The reabsorption sites in all mice were also noted. The fetuses and placentas were blotted dry and weighted individually. At the time of fetal collection, fetal body weight (g), placenta weight (g) and crown rump length in cm (length from tip of nose to root of tail) of each fetus were also noted. (Photo 3 & 4)

The Placenta and fetuses were carefully preserved and fixed in 10% buffered formalin.

Preserved and fixed samples such as placenta and fetus were collected from treated group „A‟ and control group „B‟ and processed for paraffin sectioning. Fixed tissues of these samples were dehydrated by ascending grade of alcohol (70% to rectified spirit), cleared in xylene, followed by immersion in 2 changes of wax for 15 minutes each. Block was prepared and sections were cut by Spencer‟s Rotary microtome. Sections were transformed to albumenized slides and kept on a hot plate for spreading and fixing.

The paraffinised sections were immersed in xylene for 5 -10 minute and then transfer to absolute alcohol. Hydration of the section was done through the descending grades of alcohol (95% to 70% alcohol). Kept the section under tap water for few seconds and immersed into acid alcohol and ammonia water. Staining was done by Haematoxylin and eosin stain. Dehydration of the section was done by ascending grade of alcohol (70% to absolute alcohol). Section was mounted with cover slip by using DPX for histological observation.

Observation and Results

There was no convulsion; lethargy, sleep or coma was found in mice of either group. There is also no complain of diarrhea in any of the mice of both the group.

Color of skin, fur, eyes and mucus membrane of mice were examined daily of pregnant mice of both the group but no significant changes was observed in either group.

Gain in weight of pregnant mice of group,, A‟ &,, B‟ was observed on day 3rd, 9thand 18th after conception, but weight gain in mice of any group was not found significant (table no.2).

Table No. 2: Mean weight of pregnant mice on the day of conception 3rd, 9th and 18th.

Group	Day 0	Day 3	Day 9	Day 18
A	25.4±1.94	24.6±2.30	28.8±2.58	37.0±3.53
B	24.8±0.83	24.0±0.70	28.6±1.14	37.8±1.78
Intergroup Comparison One Way ANOVA	F=0.171 p=0.845*	F=0.209 p=0.814*	F=0.667 p=0.531*	F = 0.260 p=0.775*

*NS=non significant (p>0.05)

After laprotomy of pregnant mice on 18th day, 46 live fetuses, 4 resorption and 1 dead fetus were from Group A and 42 live fetuses from Group B were collected. After statistical analysis, the mean of number of fetuses per mice in any groups was not observed significant than other [table-3].

Table No. 3: No. of resorptions, dead and live fetuses extracted from the pregnant mice belonging to Group A & B-

Groups	Days of treatment (SKCR/DW)	Total no of experimental female mice	Resorption	No. of dead fetuses	No. of live fetuses	Intergroup comparison One way Anova
A	5	5	4	1	46 Σ=5.24±2.90	F=0.973 P=0.381 A vs. B (NS)
B	5	5	0	0	42 Σ=5.05±2.87	

NS=non significant (p>0.05)

Mean weight, placental weight and length of fetuses of Group A and B on day 18th day was taken. On statistical analysis, it was found that fetal weight, placental weight and length of Group A fetuses were found significantly less as compared to Group B fetuses (table no. 4).

Table No. 4: Effect of SKCR drug on weight, placental weight and length of 18 days old fetuses.

Group	Wt. of Fetus (g)	Wt. of Placenta (g)	Length of Fetus (mm)
A (n=46)	1.030±0.158	0.0775± 0.010	33.80±2.621
B (n=42)	1.194±0.127	0.0861± 0.024	35.98±1.932
Intergroup Comparison One Way ANOVA	F=17.68 p=0.000	F=14.438 p=0.022	F=11.750 p=0.000
Post Hoc Test (LSD) (AvsB) Significant pairs	p<0.001	p<0.05	p<0.001

Insignificant= p>0.05, Significant=p<0.05, Highly significant =p<0.001

Histological Finding (H &E 400x)

Cerebral cortex

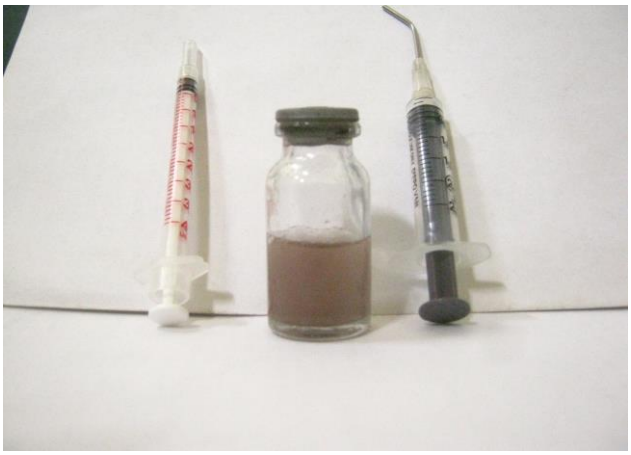
In treated Group A, mice brain cortex showing massive degeneration in thalamic region and hemorrhage in third ventricle (slide1). Mice brain also showing neuronal degeneration in the thalamic region of the periventricular area (slide 2), while white matter of cerebral cortex showing the fiber architecture sandwiched with neurons (Slide 3) and cerebral cortex showing the lateral ventricles and adequate neuronal population with the choroid plexus in the ventricular region of the fetuses of group B. (Slide 4).

Lungs

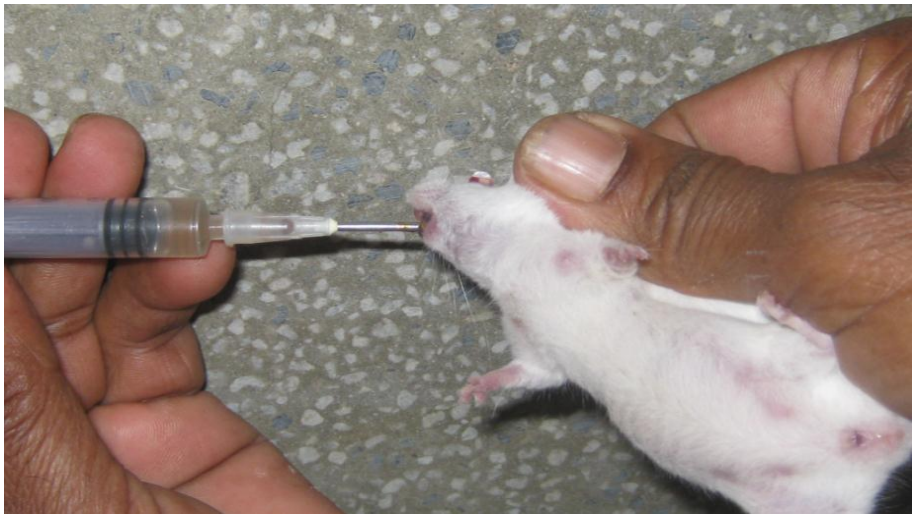
The fetuses of Group A mice showing variable density of lung tissue centrally placed edematous zone and apoptosis. (Slide 5). Lung parenchyma is also showing undifferentiated parenchymal tissues and cartilage cells, which are not differentiated. (Slide 6) while the fetuses of Group B, lungs parenchyma showing the normal cytoarchitecture (slide 7) and homogenous cell distribution (Slide 8).

Liver

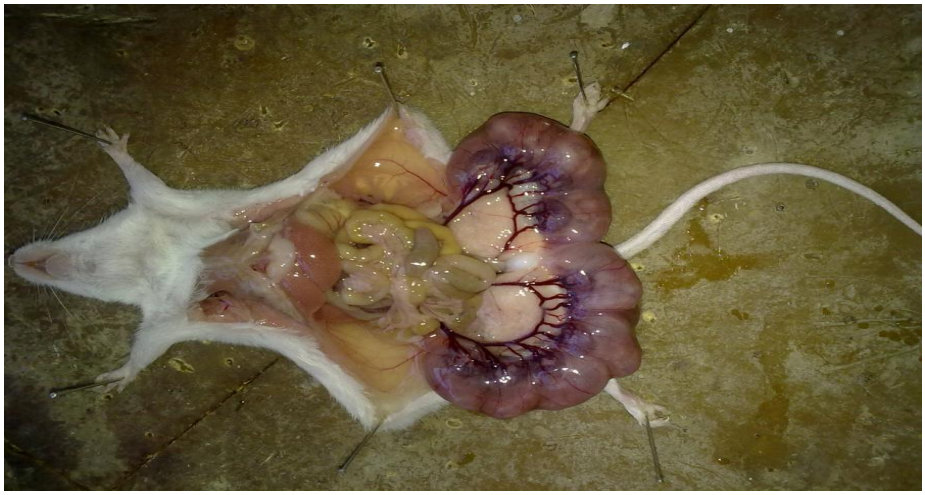
In Group A fetuses, parenchyma of the liver is showing massive edema and scanty kuffer cells. (Slide 9) and hepatocytes are reduced in number with massive edema in the pericellular region and normal cytoarchitecture is disturbed (Slide 10) while in Group B fetuses, liver parenchyma is showing normal distribution of hepatocytes and kuffer cells. (Slide 11).



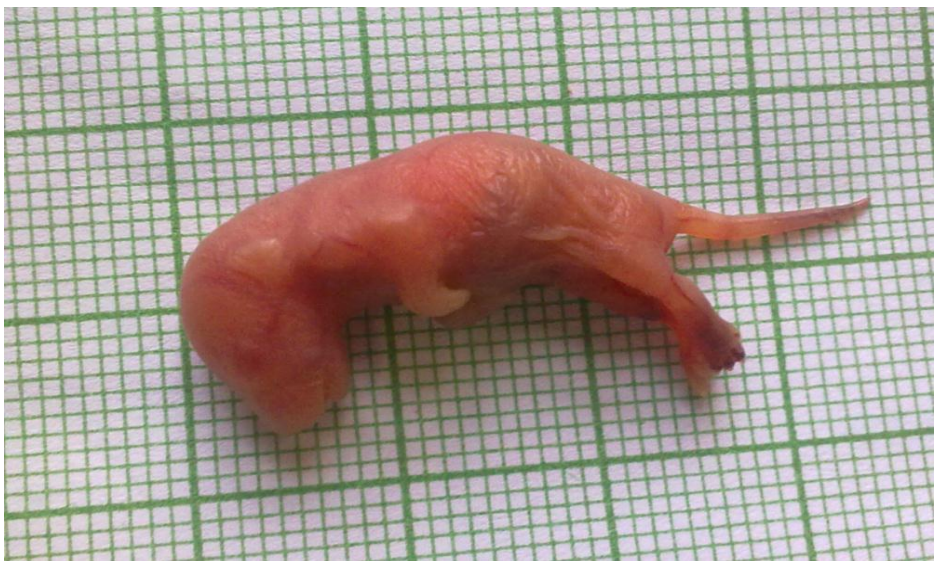
Prepared Drug with Needle(Photo 0)



Drug Installation **(Photo 1)**

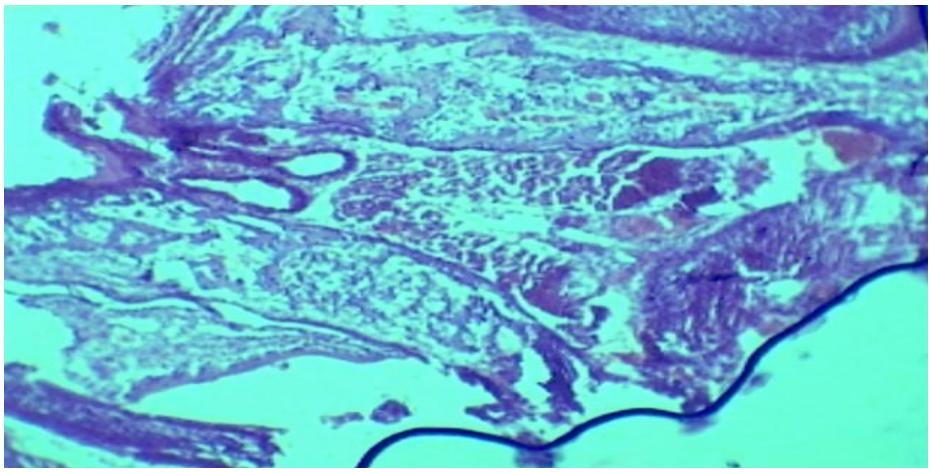


Mice dissection **(Photo 2)**

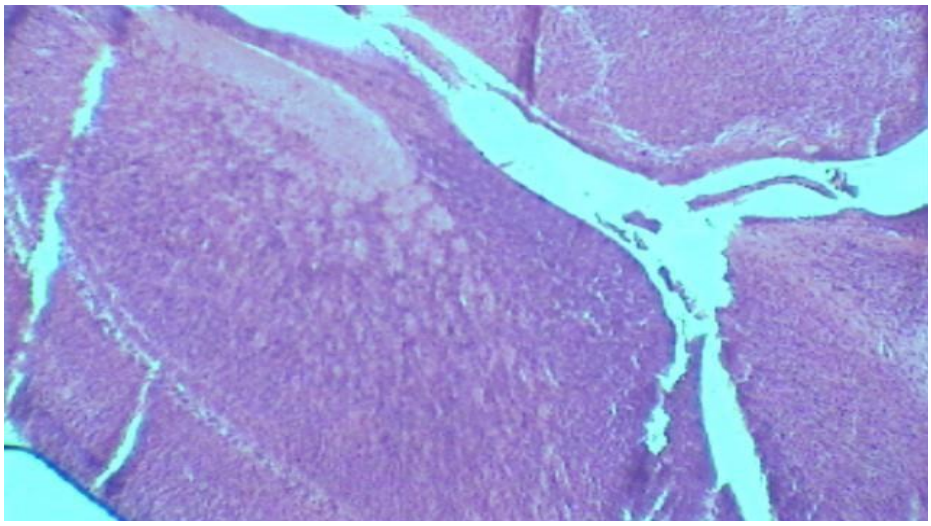


Length of fetus **(Photo 3)**

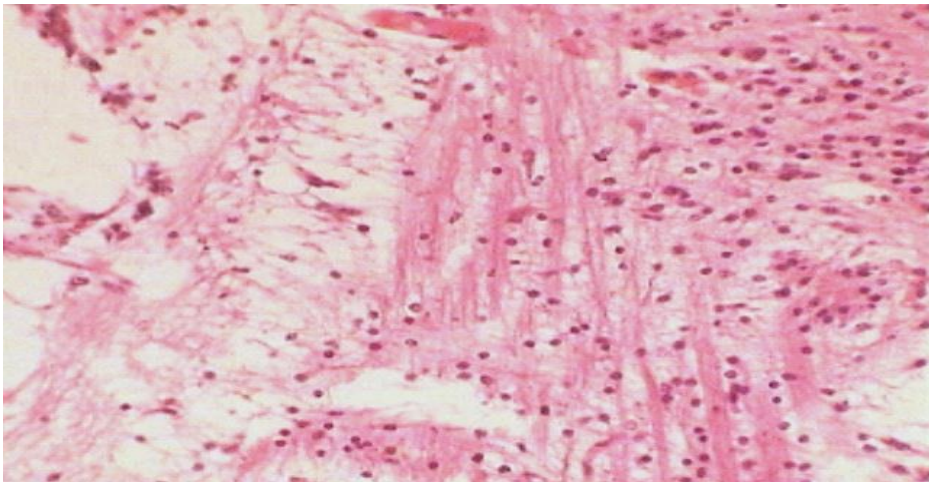
Weight of Fetus **(Photo 4)**



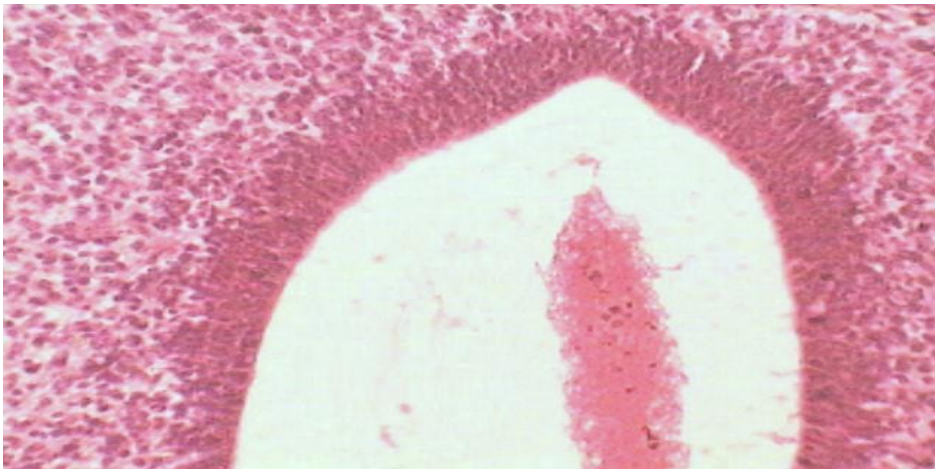
Slide 1: Treated Group A(16mg/kg/d), mice brain cortex showing massive degeneration in thalamic region and hemorrhage in third ventricle Slide.



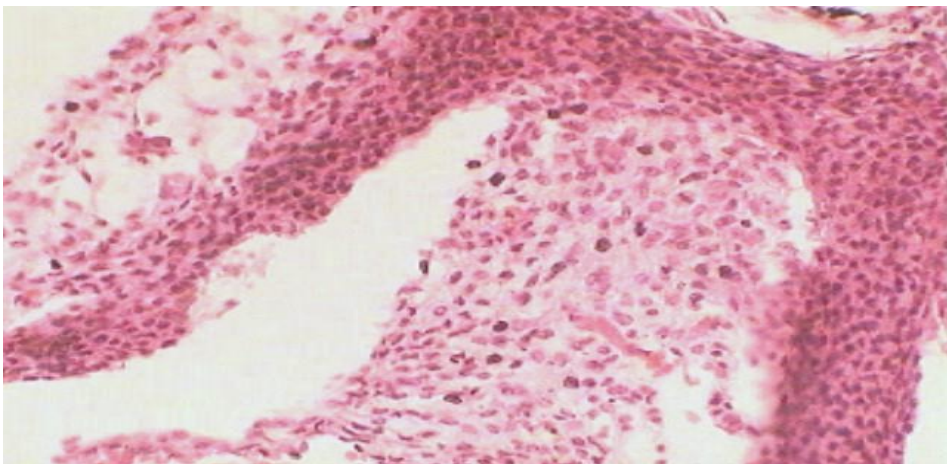
Slide 2: Treated group A(16mg/kg/d), mice brain also showing neuronal degeneration in the thalamic region of the periventricular area



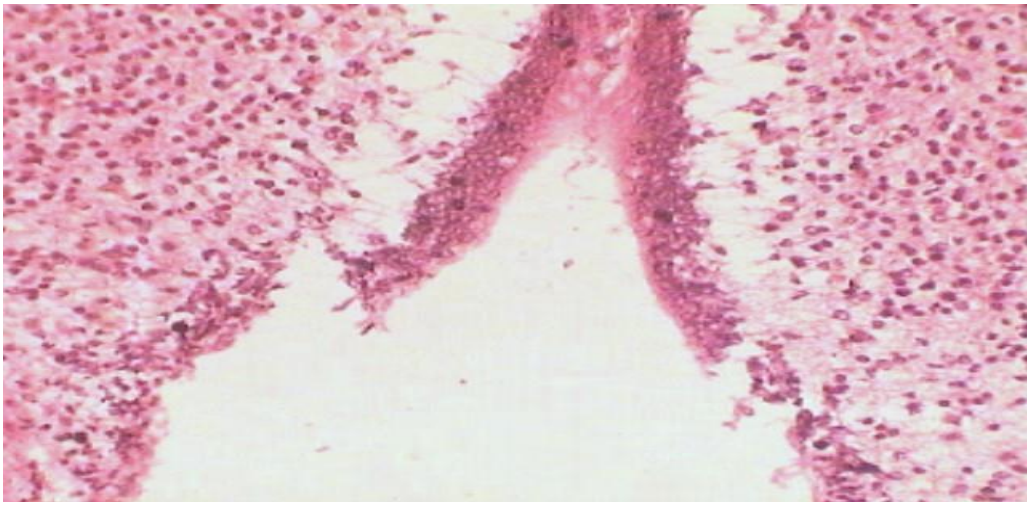
Group B (control): Control white matter of cerebral cortex showing the fiber architecture sandwiched with neurons. (H & E 400x) [Slide-3]



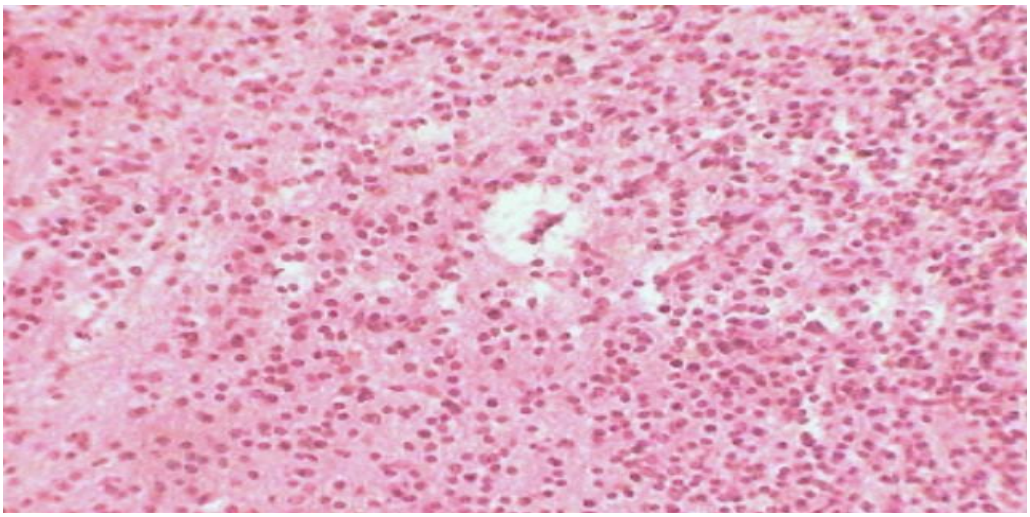
Group B: Control cerebral cortex showing the lateral ventricle and adeqauate neuronal population with the choroid plexus in the ventricular region. (H & E 400x) [Slide-4]



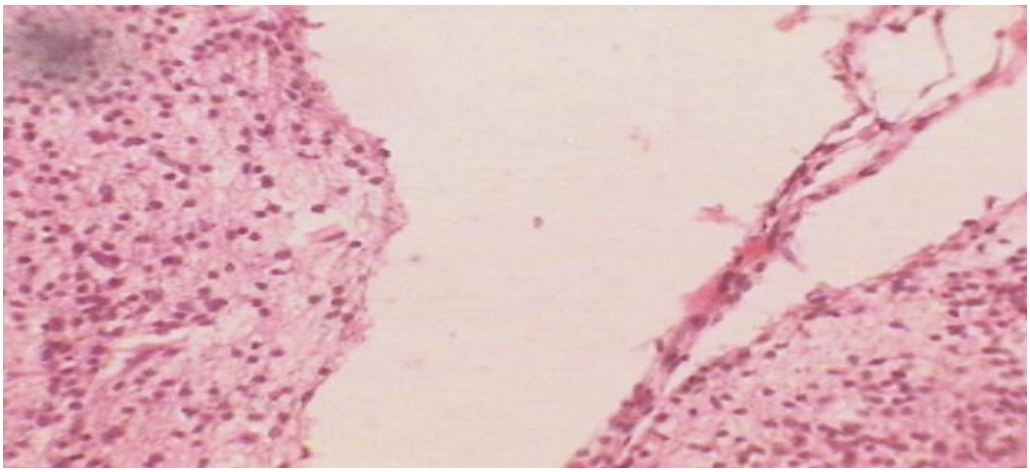
Group A: (16mg/kg/d SKCR): Treated lungs showing variable density of lung tissue and centrally placed cells showing the Oedematous zone and apoptosis. (H & E 400x) [Slide-5]



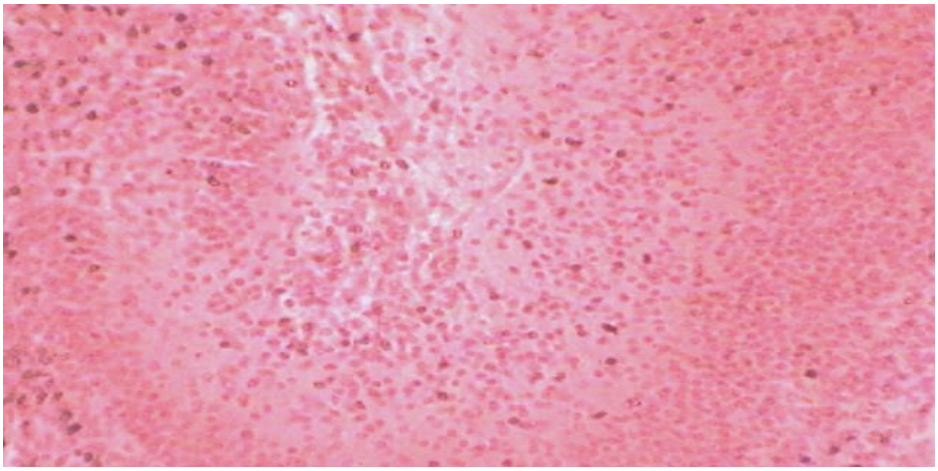
Group A: (16mg/kg/d SKCR): Treated lungs parenchymal showing undifferentiated parenchymal and the cartilage cells were not differentiated. (H & E 400x)[Slide-6]



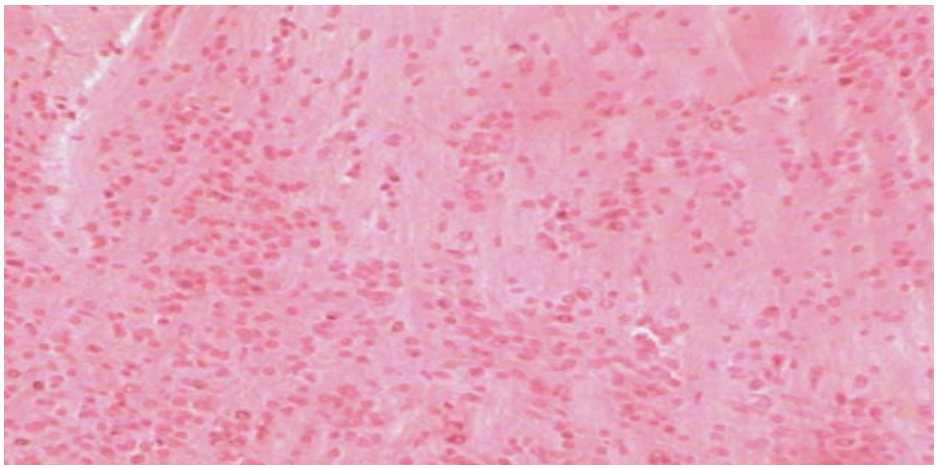
Group B: Control lung showing the normal cytoarchitectonics. (H & E 400x) [Slide-7]



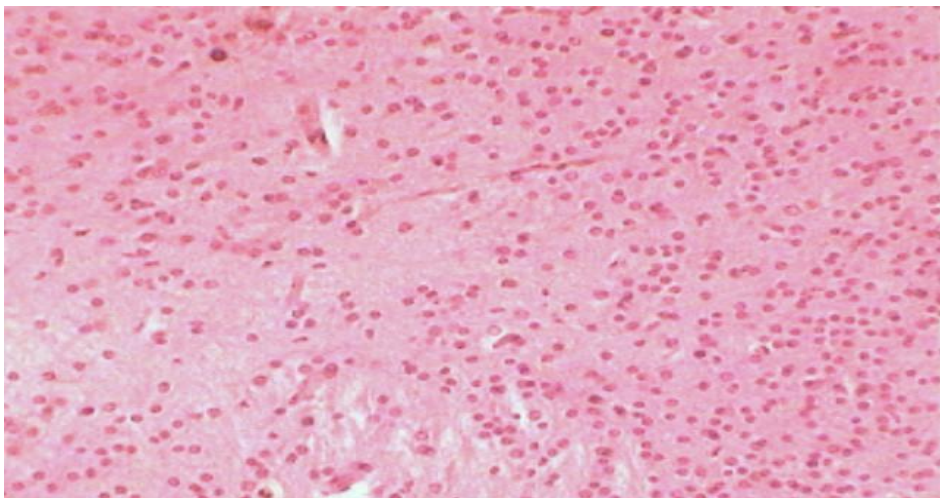
Group B: Control lung showing the homogenous cell distribution. (H & E 400x) [Slide-8]



Group A: (16mg/kg/d SKCR): Treated parenchymal of the liver showing massive oedema and scanty Kuffer cells. (H & E 400x)[Slide-9]



Group A: (16mg/kg/d SKCR): Treated hepatocytes are reduced in number with massive oedematous zone in the pericellular region and normal cytoarchitectonic is disturbed (H & E 400x) [Slide-10]



Group B: Control hepatocytes showing the normal cell distribution (H & E 400x) [slide-11]

DISCUSSION

In the present study, it was observed that statistically gain in weight by the pregnant mice and mean number of fetus delivered by mice, are not found significant [p>0.05] on intergroup comparison (table no 2, 3) while the resorption were found in only group A not in control Group B. On macroscopic examination, no gross morphological abnormality are observed in any fetus of group A & B. All these findings suggest that the drug SKCR does not have the effect to change the gross morphology of fetus when fed to the pregnant mice during the most vulnerable period [5-9 days].

Intergroup correlation of mean weight and length of fetuses, delivered by each mice, was recorded just after extraction and found that growth in fetuses of pregnant mice of group-B was higher than group-A (table no. 4). These findings suggest that there is significant growth retardation in terms of weight and Length of fetuses of mice of Group-A. The mean weight of the placenta of group-B was higher from group-A (table no. 4).

In the present study, histological finding of treated groups [A] has also shown reduction in neuronal population, massive edema, scanty Kuffer cells, degeneration, apoptosis etc. in the tissues of various organs in different slides.

These embryo toxic effects on multiple organs seem due to presence of free Hg or toxic mercurial salt present in processed SKCR drug, collected from the market.

CONCLUSION

On the basis of this study, it can be concluded that Shwasa-Kasa-Chintamani-Rasa [SKCR], a herbo-mineral drug, available in the market, is embryo toxic and should not be used during pregnancy and younger children until it is not proved safer for the human. This drug may be kept under Category - C.

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