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TOXICITY STUDY OF *KUSHTHAVACHADI CHOORN* INHALATION IN ALBINO MICE

Vd. Swati Vinayak Gaikwad[1]* and Dr. Sujata D. Kadam[2]

[1]*Ph.D Scholar, Prasutitantra Streerog Department, Research Institute of Health Science and Management, Pune.

[2]Professor, HOD, Prasutitantra Streerog Department. Dean- AIIA, Delhi.

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***Corresponding Author**
Vd. Swati Vinayak Gaikwad
Ph.D Scholar, Prasutitantra Streerog Department.
Research Institute of Health Science and Management, Pune.

ABSTRACT

Background: Prolonged labor due to uterine dystocia is one of the important reasons for pregnancy complications. *Kushthavachadi choorn* inhalation has been advised in the *charak samhita* for enhancement of uterine contractions in uterine force insufficiency.[1] Very little research is being carried out during pregnancy and labour with a fear to harm the foetus and threat of legal liability. The inhalation of this *choorn* is advised during labor for enhancing uterine contraction. All the drugs in *kushthavachadi choorn* are used orally since ages. Fetal growth and maturity is ensured at full term. To rule out the inhalation toxicity of *kushthavachadi choorn*, animal study for inhalation toxicity of *kushthavachadi choorn* was carried out in pregnant Albino mice. **Method**-This was a controlled study where one group of pregnant Albino mice was exposed to the drug *kushthavachadi choorn* in a chamber with the help of nebulizer. These mice were keenly observed before and after exposed to the drug. **Result:** There were no sign of discomfort or behavioral changes during exposure to fumes of *kushthavachadi choorn*, as well as for next 24 hours after exposure to *kushthavachadi choorn*. All the mice in trial group delivered 24 hours earlier to that of the control group. They delivered within 24 hours of exposure to *Kushthavachadi choorn* inhalation, indicates that this inhalation has stimulated the uterus, that has lead to the delivery of these mice. **Conclusion:** Animal study reveals there is no inhalation toxicity of *Kushthavachadi choorn* in Albino mice. Kushthavachadi choorn inhalation has stimulated the uterus. It has uterine contractile activity.

KEYWORDS: Dystocia, *kushthavachadi choorn*, inhalation toxicity, parturation.

INTRODUCTION OF ANIMAL STUDY

Pregnant state of woman causes pregnancy disorders that lead to compromised mother's health and their babies also. There are significant physiological changes in pregnancy, including nearly doubling of maternal blood volume and alteration in binding proteins, the pharmacokinetics and efficacy of drugs are by enlarged unknown. Still today pregnant woman are largely excluded from clinical research with a fear to harm the foetus and threat of legal liability. Responsible inclusion of pregnant woman in clinical research, based on ethical reasons and medical needs are promoted by NIH (National Institute of Health). Thus, the pregnant women are re-classified from vulnerable population to that of medically complex population, necessitating special scientific and ethical consideration. Pregnant woman are an especially dynamic subset of woman. NIH is an important U.S Health Agency, devoted for medical research and research on woman's health. ORWH (Office of Research on Woman's Health), it's a component of NIH, which promotes ethical consideration of research in pregnant women. In *charak samhita* the drug *kushthavachadi choorn* has been advised as a drug of choice to enhance uterine contractions in case of prolonged labour.[1] Similar drug administration has been advised in *kashyap samhita- Jatisutriya* chapter.[2] In the study of *kushthavachadi choorn*, the drug is administered after the full development of foetus. Hence, there is no risk as to cause any interference with growth and maturity of foetus. Also the mothers have an open choice to get all the necessary and emergency treatment as and when required. Thus, only the issue of inhalation toxicity remained. The mucosa of pregnant woman is delicate as compared to non pregnant state. Thus, the chances of inhalation toxicity prevailed.

Hence, an Animal Study to rule out inhalation toxicity of *kushthavachadi choorn* was carried out at NTC (National Toxicity Centre, Pune). While carrying out the toxicity study, the pregnant mice were exposed to the drug *Kushthavachadi choorn*. The mice being pregnant, it was possible to observe the effect of the exposure of this drug on pregnancy outcome as well as its effect on induction of labour, which brought to our notice about its action on the uterus.

Aim: To study the inhalation toxicity of *Kushthavachadi choorn* inhalation in pregnant Albino mice.

Objective: 1) To assess the effect of *kushthavachadi choorn* inhalation on outcome of pregnancy.

2) To assess whether the drug *kushthavachadi choorn* has any effect on the induction of Labour and the uterine activity.

Ethics Committee: With an aim of Animal Welfare, clearance from institutional ethics committee for toxicity study was obtained.

MATERIALS AND METHODS

Test Method Principal

The *kushthavachadi choorn* is advised to be administered to women's during the labour process by inhalation in powder form to augment the labour process by enhancing uterine contractions. This drug though advised in the text is not routinely used for inhalation purpose. It was necessary to carry out inhalation toxicity of this drug before using it in pregnant women. Mucosa of pregnant mice is similar to that of pregnant women. Hence, to carry out inhalation toxicity study of *kushthavachadi choorn* pregnant Albino mice were exposed to the aerosol of this drug by fumes administered in a chamber with the help of a nebulizer.

Formulation

Kushthavachadi Choorn is an *Ayurvedic* herbal preparation mentioned in *Charak samhita*, which is intended to be used for the treatment of augmentation of labour by inhalation.[1]

Drug Preparation

Table 1: Ingredients with their Latin Name of *kushthavachadi* choorn.[3]

Sr. No.	Name of the Drug	Latin name
1	*Kushtha*	Saussurea lappa
2	*Ela*	Amomum subulatum
3	*Langli*	Gloriosa suberba
4	*Vacha*	Acorus calamus
5	*Chitrak*	Plumbago zeylanica
6	*Chirbilwa*	Holoptelea integrifolia
7	*Chavya*	Piper retrofractum



Fig 1: Saussurea lappa. Fig 2: Amomum subulatum. Fig 3: Gloriosa suberba.



Fig 4: Acorus calamus. Fig 5: Plumbago zeylanica. Fig 6: Holoptelea integrifolia.


Fig 7: Piper retrofractum. **Fig 8: *Kushthavachadi Choorn*.**

Collection of Raw Drugs

Raw Drugs were collected from the authentic source – authorized pharmacy.

Chirbilwa was collected from botanical garden.

Part of the plant used in the drug

Roots of *Kushtha, Vacha, Langli and Chitrak* were used.

Dried fruits of *Ela, Chavya and Chirbilw* were used.

This drug was used for inhalation. The description of all these drugs in the text is given on the basis that the drug is ingested in the digestive system, and its effects on various systems are described. The effects of these drugs on inhalation have to be understood and predicted from its Guna, Virya, Vipak etc.

Table 2: Showing pharmacodynamic properties of ingredients of *Kushthavachadi choorn*.

Name of the drug	Guna	Rasa	Virya	Vipak	Action on Doshas	Action on Systems
Kushtha[4]	Laghu Ruksha Tiksha	Tikta Katu Madhur	Ushna	Katu	Vata, Kapha shamak	Stimulates the nervous system.
Ela[5]	Laghu Ruksha	Katu Tikta	Ushna	Katu	Kapha, Vata shamak	Stimulates Cardiactivity
Langli[6,7]	Laghu Tikshna	Katu Tikta	Ushna	Katu	Kaph, Vata shamak	Uterine contractile activity
Vacha[8]	Laghu Tikshna	Katu Tikta	Ushna	Katu	Kaph, Vata shamak	Medhya acts on central N.C. as stimulant
Chitrak[9]	Laghu Ruksha Tikshna	Katu	Ushna	Katu	Kaph, Vata shamak	Stimulates N.S. uterine contractile activity
Chirbilwa[10]	Laghu Ruksha	Tikta Kashay	Ushna	Katu	Kapha, Pitta Shamak	———
Chavya[11]	Laghu Ruksha	Katu	Ushna	Katu	Kaph, Vata shamak	———

Authentication and Standardization of Raw Drug and Finished Product

The raw drugs so collected were given for identification and authentication at Agarkar institute, Pune. After the drugs were identified, the drug standardization was carried out in C.P.G.S. & R.A. Tilak Ayurved Mahavidyalaya, Pune. After the drugs were authenticated and standardized they were taken for drug preparation. This drug was prepared in summer season to keep it dry and protect from moisture. The finished product was also standardized at C.P.G.S. & R.A. Tilak Ayurved Mahavidyalaya, Pune.

Method of Preparation of *Kushthavachadi Choorn*

Fine powder of each drug was separately made in a mixer grinder with a mesh value of less than 100.

Before putting the drug in mixer grinder the coarse powder of these drugs has to be made in case of *Kushtha, Vacha, Langli* and *chitrak.*

The fruits of *Ela, Chavya* and *Chirbilwa* are soft and can be grinded easily. After obtaining the fine powder with mesh value less than 100.

All these fine powders were mixed together in same quantity. This mixture was named as

Kushthavachadi Choorn.

Test Item Name – *Kushthavachadi Choorn.*

Physical Appearance – Brown colored fine powder.

Vehicle Details: This choorn was to be used for inhalation purpose in pregnant women during labour. The use being in a very limited quantity and a short period of time it was anticipated that not more than 15gm choorn will be used in the whole process.

Thus, assumed human dose = 15 gm = 15000mg. (For an average 70 kg human.)

Dose conversion factor = 0.0026 (for human to mice, on the basis of surface area.)

Hence, the dose in animal is 15000 × 0.0026 = 39 mg (for average 20 gm mice.)

The mice are of different weights, so, we convert it to mg / kg.

i. e. 39 × 1000 ÷ 20 = 1950, approximately 2000 mg/ kg.

Hence, 2000 mg of drug has to be dissolved in 10 ml H_2O for nebulisation purpose.

So, 20 gm of drug was dissolved in one liter of water to prepare an aerosol. This solution was put in the nebulizer and the fumes of the aerosol were sent into the chamber where six mice of study group at 19^{th} day of their pregnancy were kept.

Inhalation Chamber: An acrylic chamber of 20 cm × 20 cm × 20 cm was used.

Test System Details

Species – Albino mice.

Sex – Female.

No. of animals used – 12 adult Albino mice.

Source – Animal Breeding and Experimental Facility of National Toxicity Centre,

Apte Research Foundation, Vadgaon, Khurd, Pune – 411041

Maharashtra, India.

Experimental Procedure

Location of Study

The study was carried out at National Toxicology Centre, Apte Research Foundation, Vadgaon, Khurd, Pune – 411041, Maharashtra.

During study all animals were maintained in Animal experimental room at Animal House facility in the centre itself.

Housing of Animals

1. **Temperature**: They were kept at 22^0 C ± 3^0C room temperature. Humidity 50 – 60 %. Illumination cycle was set to 12 hours light and 12 hours dark.
2. **Sanitation**: Cages and bedding materials were changed at least twice in a week and water bottles were changed daily. Each day, the floor of the experimental room was swept and all the worktops and floors were mopped with disinfectant solution.
3. **Husbandry Practices**: All the animals were given complete pelleted food prepared at the institution without soya products. Aqua Guard drinking water was supplied at labitum.

Matting of mice

For this study minimum of 12 Albino Mice were selected and made pregnant by mating. The mice were kept together for mating as 3 females and one male in single cage. To confirm their mating, their vaginal smears were taken and pregnancy was confirmed.

Inclusion Criteria

1. Six to eight weeks of adult Pregnant mice of weight more than 50gm but less than 60gmbreed in National Toxicity Center were only included in the study.

Exclusion Criteria

1. Albino mice in non pregnant state.
2. Albino mice breed in place other than NTC.

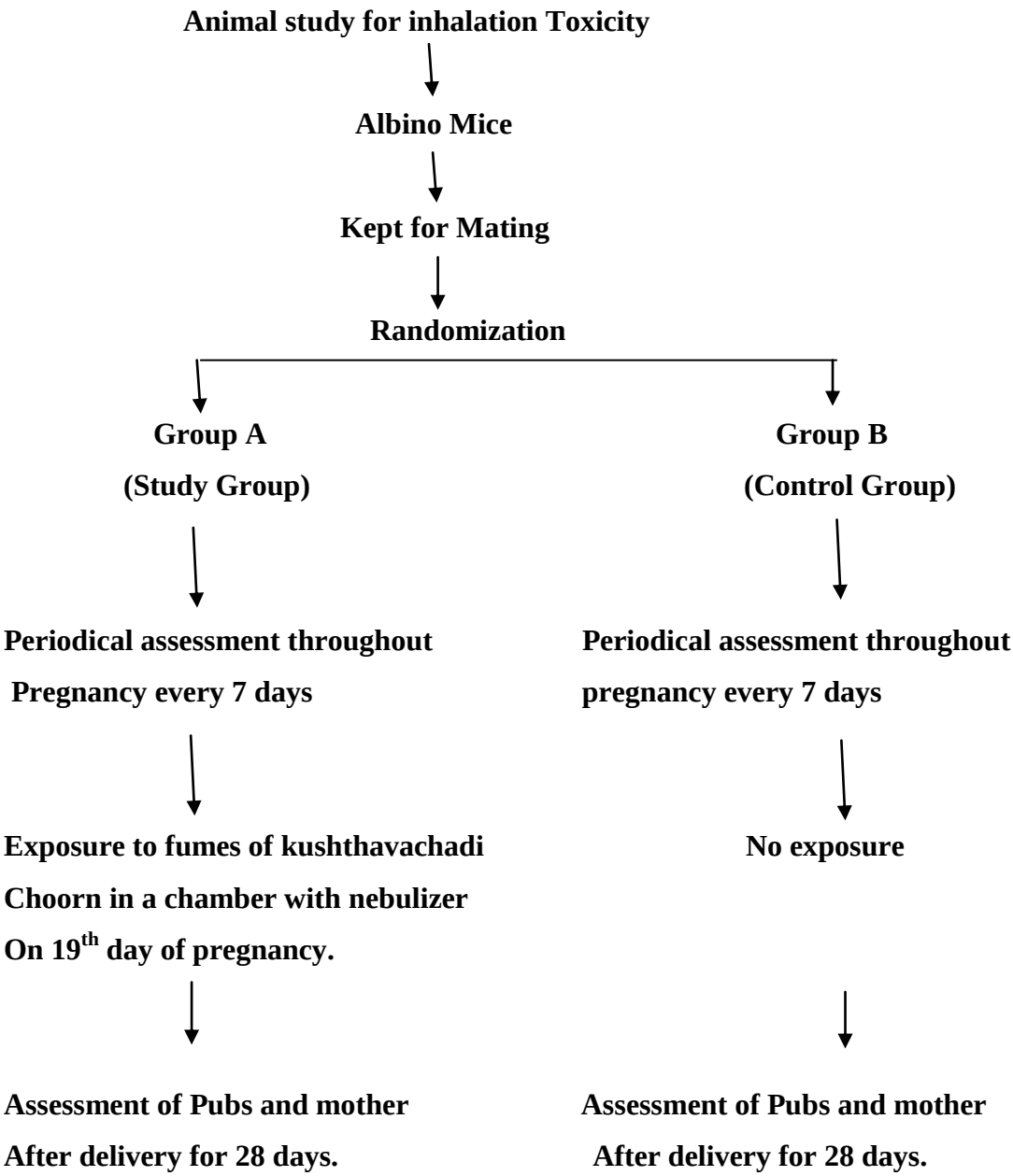
Study period

From day One of pregnancy confirmation till 28^{th} day after delivery.

Study population

This being an animal study the study population was pregnant Albino mice.

Study Design



Grouping

These mice were randomly divided into two groups. Group 'A' study group and Group 'B' control group.

In study Group these mice were to be exposed to *Kushthavachadi choorn* inhalation to carry out inhalation toxicity study.

Group B was given routine care and no intervention was done.

Route of Administration of Drug

Inhalation through the nose with the help of a chamber with a nebulizer was used. The mucosa of pregnant Albino Mice resembles the mucosa of pregnant woman. The *Kushthavachadi Choorn* inhalation toxicity was to be studied. Hence, route of administration, 'inhalation' was selected.

Experiment, Treatment and Duration

The mice normally deliver on 21st day of pregnancy. This drug is known to have its effect on uterine contraction. The inhalation toxicity was to be studied on pregnant mice; hence it was decided to expose the mice, prior to their delivery at term pregnancy. Therefore mice in study group, Group 'A' were exposed to the fumes of aerosol of drug *Kushthavachadi Choorn* on 19th day of pregnancy.

The group 'B' control group was given routine care and keenly observed. The solution of *Kushthavachadi Choorn* prepared was put in the nebulizer and the fumes were sent into the chamber with six mice of study group on 19th day of their pregnancy. The nebulisation was started at 11am and inhalation was given for two minutes. There after the nebulisation was stopped for complete ten minutes. The mice were keenly monitored and observations noted for their behavior during the exposure and the rest period as stated below. There after again an exposure for two minutes was given. In this way ten episodes of exposure at an interval of 10 minutes were carried out in a span of two hours i.e. till 1pm.

Fig 9: Chamber.

Fig 10: Chamber with Nebuliser.

Fig 11: Animal Cages.

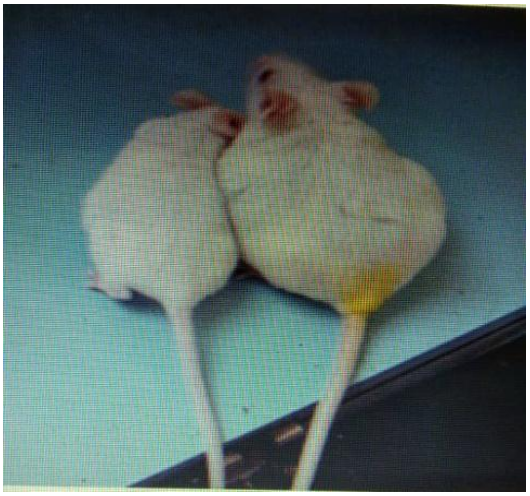


Fig 12: Figure showing Non-Pregnant (left) And pregnant (right) Mice.

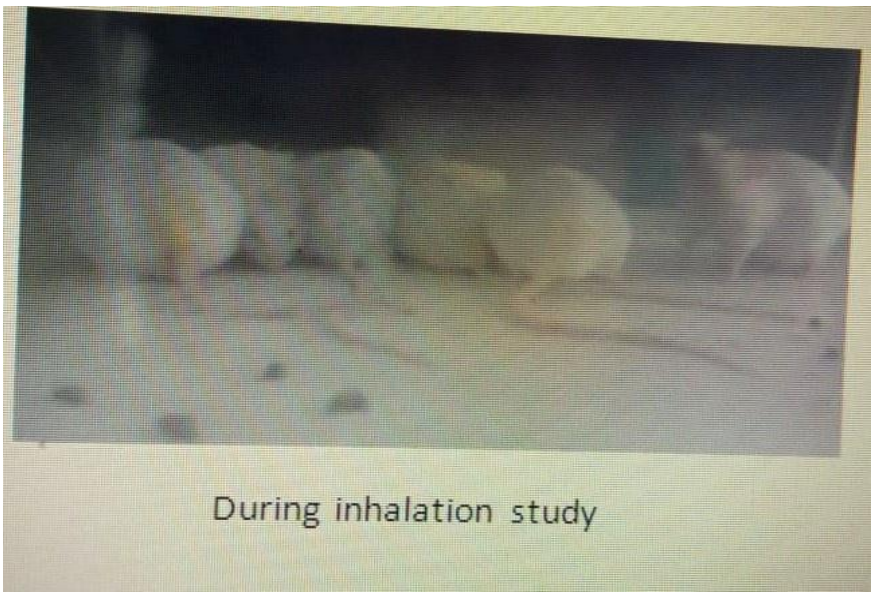


Fig 13: Mice during inhalation of *Kushthavachadi Choorn*.

OBSERVATIONS

The pregnant Albino mice, in study group, i.e. Group 'A', after exposure to the fumes of *Kushthavachadi Choorn,* following observations were carried out in next 24 Hours and every 7^{th} day after delivery till 28^{th} day after delivery.

Table 3: Signs assessed for clinical observations.

Sr. no.	Clinical Observation	Observed Signs						
1	Respiratory system	Dyspnoea	Nasal discharge	Inflammation	Apneoa	Cynosis	Tachypnoea	Asphyxia
2	Digestive system	Salivation	Emesis	Diarrhoea	-	-	-	-
3	Motor activity	Unusual locomotion	Tremors	Sedation	Coma	-	-	--
4	Convulsions	Convulsion	Clonic Convulsion	Tonic Convulsion	Tonic Clonic Convulsion	-	-	-
5	Ocular	Lacrimation	Conjunctivitis	Opacity	-	-	-	-
6	cardiovascular	Bradicardia	Tachycardia	Arrhythmia	-	-	-	-
7	Urinary system	Normal Urination	Haematuria	-	-	-	-	-
8	Others	Weight	-	-	-	-	-	-

Observation in Group 'A'

1. There were no sign of discomfort or behavioral changes during exposure to fumes of *kushthavachadi choorn*, as well as for next 24 hours, after exposure to *kushthavachadi choorn*.
2. There were no excessive nasal discharges or inflammation seen at the nasal region in all the 6 mice.
3. There were no significant signs of adverse reaction regarding the systemic observations as mentioned above, in all the 6 mice.
4. There was no significant change observed in consumption of food.
5. All the 6 mice exposed to *kushthavachadi choorn* fumes inhalation, delivered on 20th day; i.e. the delivery occurred within 24 hours after the exposure to the drug.

Observation in Group 'B'

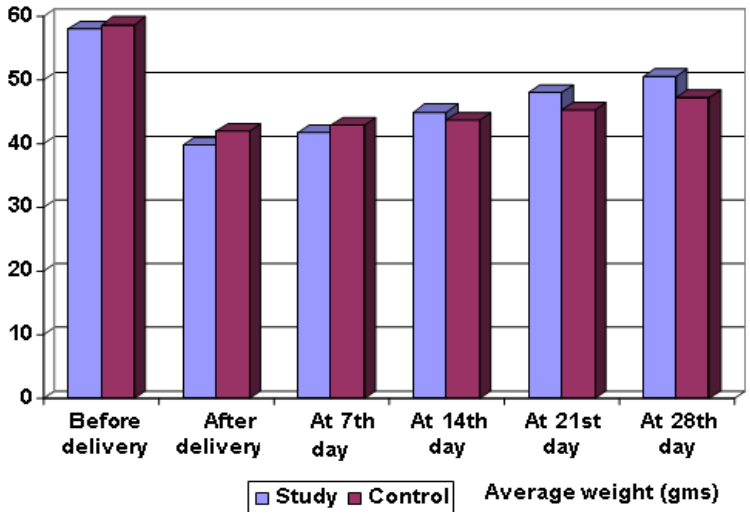
1. All the mice in control group delivered on 21st day.

Statistics

Unpaired 'T' test was applied for comparison of both groups.

Table 4.1: Comparison of weight of mice before delivery, after delivery, 7th day, 14th day, 21st day and 28th day after delivery in study Group 'A' and control Group 'B'.

Weight (gm)	Study (n=6)		Control (n=6)		t Value	P Value
	Mean	SD	Mean	SD		
Before delivery	58.083	9.9419	58.750	6.0312	0.14	0.89
After delivery Day 0	39.833	6.1536	42.000	3.9875	0.72	0.49
At 7th day	41.833	5.7067	42.917	4.6089	0.36	0.72
At 14th day	45.000	5.9666	43.750	3.9969	0.43	0.68
At 21st day	48.000	5.9582	45.333	3.5870	0.94	0.37
At 28th day	50.550	6.4059	47.333	3.2965	1.09	0.30

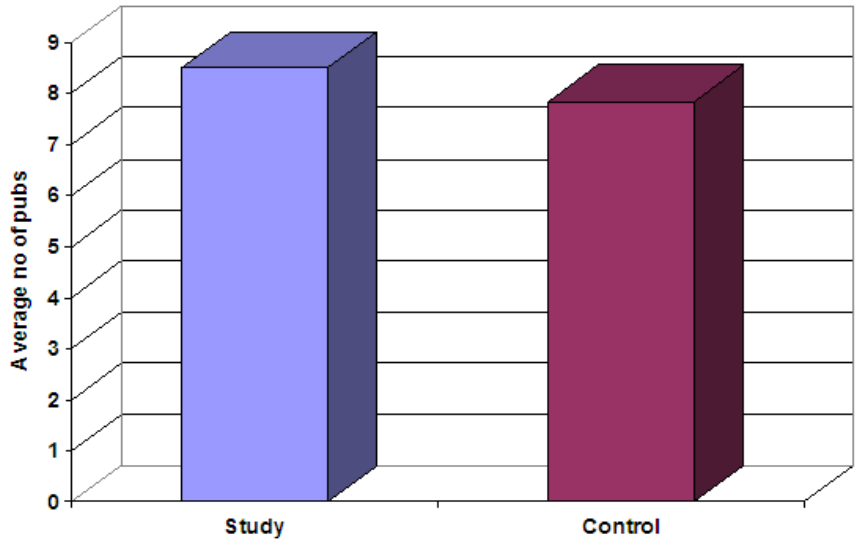


Graph 1: Bar diagram showing comparison of weight before delivery, after delivery, 7th day, 14th day, 21st day and 28th day after delivery in study group and control group.

The mean weights of mice in study group and control group before delivery, after delivery, 7th day, 14th day, 21st day, 28th day after delivery shows no significant difference as p value is greater than 0.05.

Table 4.2: Comparison of number of pubs in study and control group.

Parameter	Study (n=6)		Control (n=6)		t Value	P Value
	Mean	SD	Mean	SD		
No of pubs	8.50	4.037	7.83	1.722	0.37	0.72



Graph II: Bar diagram showing comparison of number of pubs in study and control group.

The average number of pubs delivered in both the groups is same and shows no significant difference as p value is greater than 0.05.

Table 4.3.: Observations of delivery of mice in Study Group- Group 'A', after exposure to *Kushthavachadi choorn*.

Mice	Day of pregnancy at the time of inhalation	Day of delivery of mice, after exposure to the drug	No. of Pubs delivered	Adverse Reaction
1	19th	20th	6	Nil
2	19th	20th	8	Nil
3	19th	20th	15	Nil
4	19th	20th	3	Nil
5	19th	20th	10	Nil
6	19th	20th	6	Nil

Table 4.4. Observations of delivery of mice in Control Group- Group 'B'.

Mice	Day of delivery of mice	No. of Pubs delivered	Reaction, if any
1	21st	6	Nil
2	21st	6	Nil
3	21st	7	Nil
4	21st	9	Nil
5	21st	10	Nil
6	21st	9	Nil

The Mice were observed every 7th day during pregnancy till delivery, after delivery the mice and the pubs were observed for a period of 28days after delivery. Weight record of mice and the pubs was recorded after delivery on 7th day, 14th day, 21st day, 28th day of delivery.

RESULT

1. There is no acute toxicity of *Kushthavachadi choorn* inhalation in Albino mice.
2. There is no inhalation toxicity of *Kushthavachadi choorn* in Albino mice.
3. All the mice in trial group delivered 24 hours earlier to that of the control group. Also they were seen to be delivered within 24 hours of exposure to *Kushthavachadi choorn* inhalation, indicates that this inhalation has stimulated the uterus, that has lead to the delivery of these mice.

DISCUSSION

1. All the drugs in the kushthavachadi choorn are consumed orally since ages. There is no toxic effect of these drug on oral consumption in proper doses. This drug was to be used for inhalation purpose in pregnant women. The risk of inhalation toxicity could not be ruled out as the drugs are *Ushna, Tikshna;* hence, toxicity study for inhalation was necessary.

2. Only behavioral, local and systemic observations of the animal was observed, histological examination be carried out in case of demise of the animal as the drug was not ingested in the digestive system. The dose of drug used being very small in quantity systemic interference is negligible. There were no sign of discomfort or behavioral changes during exposure to the fumes of *kushthavachadi choorn*, as well as for next 24 hours, after exposure to *kushthavachadi choorn*. There was no excessive nasal discharge or inflammation seen at the nasal region in all the 6 mice. There were no significant signs of adverse reaction regarding the systemic observations as mentioned above, in all the 6 mice. There was no significant change observed in consumption of food. Though the drug is *Ushna, Tikshna* there was no adverse reaction seen because the drug is used in very less quantity, each inhalation was administered for two minutes which is not potential to produce irritability there is a gap of ten minutes after each exposure which enables the mice to get accustomed to the fumes of the drug. Thus we understand the safety of the use of this drug.

3. All the 6 mice exposed to *kushthavachadi choorn* fumes inhalation, delivered on 20th day; i.e. the delivery occurred within 24 hours after the exposure to the drug. The normal time period for the delivery of mice is 21st day after conception. But, in this Group, all the mice have delivered, one day prior to the expected date of delivery. This is not by chance as all the mice delivered on 20th day in study group. This shows *kushthavachadi choorn* has contractile effect on uterine muscles by inhalation.

The mean of average weight of mice before delivery, after delivery, every 7th day after delivery till 28th day of delivery is similar to that of control group. There was no adverse reaction of drug observed. Hence it can be stated this drug does not affect the metabolism after delivery.

The average number of pubs in both groups is similar. Excessive cannibal activity was observed in two mice in study Group. The delivery occurred pre term hence the pubs not fit for survival were cannibalized.

CONCLUSION

1. There is no inhalation toxicity of *Kushthavachadi choorn* inhalation in Albino Mice.
2. *Kushthavachadi choorn* is effective on uterine contraction.

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