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TOXICOLOGICAL STUDY OF VATAGAJENDRA SINHA RASA BY L.D.50 METHOD

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ABSTRACT

Now-a-days the mentality of professionalism is increasing. As the demand for the medicine is increasing rapidly, the pharmaceutical companies are responsible for maximum production in minimum time. Hence the quality of medicine is often compromised. Very few *vaidya*s use self-prepared medicines following norms mentioned in *granthas*. As a large number of populations depend on marketed samples, it is necessary to check their safety. In foreign countries, many articles were published stating ayurvedic drugs have metallic contents which deposit in the body leading to metal poisoning & have banned the use of ayurvedic drugs for the same [(13)]. Faulty preparation techniques or not following the norms given in our *granthas* by the pharmaceutical companies could be a major reason. Metallic particles in the medicines should be so fine as to allow its easy absorption and even excretion at cellular level. If these particles are large, its excretion is restricted. It leads to unwanted deposition of the metal which further leads to its toxicity. This is an alarming sign to re-evaluate the safety of ayurvedic drugs through toxicity studies. It is necessary to evaluate the safe dose of each rasa kalpa. All vaidyas know the therapeutic dose but they don't know the lethal dose. By knowing the lethal dose they will get a limit upto which the dose can be extended. For the same reason the topic 'Toxicological study of *Vatagajendra sinha Rasa* by L.D.50 method' has been studied.

Keywords: L.D.50 study, Rasa Kalpa, metal toxicity, drug safety.

INTRODUCTION

Visha (poison) is a substance, which enters the body, spreads rapidly and vitiates the dosha, dhatu & mala disturbing all natural and physiological functions of the body resulting in destruction of life.[(5)] Though these poisons can prove to be harmful and dangerous to life, Ayurveda has also mentioned its uses as medicines after shodhan procedure. It has been proved that the *gunas* of a formulation are enhanced if it contains *vishas*, which helps in increasing its penetration property & bio-availability by the virtue of visha gunas like sukshma, tikshna etc [(8)] For this, it is of great importance to use such preparations in proper dose as: Visha if given in proper dose acts like amruta and if amruta is given in excess, it becomes poisonous.[(8)] Drug if given in very low dose, the desired results won't be achieved and if given in very high dose, it can be hazardous. So the drug dose needs to be increased according to kala, agni, vaya, prakruti, dosh, desh of rugna, the *vaidya* should know the effective dose as well as lethal dose to avoid its acute oral toxicity, especially in case of rasakalpas which contains vishadravyas.[(2)] In today's fast moving world *vatavyadhis*

are on a high. *Ayurveda* plays a significant role in the treatment of *vatavyadhis.* Even Neurologist prefers ayurvedic medicated oil as many a times they are left with no option. As such, *Ayurveda* holds pride in the results of *vatavyadhis*. In most of the *kalpas* used in *vatavyadhis* particularly in *margavrodhajanya vatavyadhis, visha dravyas* are used for its *tikshna, ushna, sukshma* properties to overcome the *avarana*. Also, the *shoola* in *vatavyadhis* is taken care for quickly due to the *gunas* of *visha dravyas* as said earlier. *"Vatagajendrasinha Rasa"* is widely used drug in treating *vatavyadis.* Its other usage are described as

Vatagajendrasinha Rasa is said to be useful in all 80 types of *vata vyadhi*, 40 types of *pitta vikaras* as well as 20 types of *kapha vyadhi* if used skillfully. It is mainly used in *ksheenindriya*, *nashtashukra* and *agnimandya*. It can be used in geriatrics as *balya* and *vayasthapana*. It is used in *khanja, pangu, kubja, ksheen* as its action is of *mansavardhana*.It is used in the diseased as well as in healthy.[4]

Vatagajendrasinha Rasa is a herbomineral drug containing *Abhrak, Loha, Parad, Gandhak, Tamra, Naag, Tankan, Vatsanabh, Saindhav, Lavang, Hingu & Jatiphal in equal quantity & Trisughandha [Twak, Ela, Patra] ,Triphala[Amlaki, Haritaki & Bibhitak] & Jeerak in half quantity triturated in Kumari swarasa.*

In modern science the contents of this drug-*Parad, Naag, Tamra* are included in metallic irritant poisons and *Vatsanabha* (Aconitum ferox) in cardiac poison.[11]

Ayurvedacharya has included Parad, Naag, Tamra in "khanija *visha*". Sushrutacharya has included Vatsanabha in "Mahavisha" [1].

Articles published in foreign countries, stating ayurvedic drugs have metallic contents which deposit in the body leading to metal poisoning is an alarming sign to re-evaluate the safety of ayurvedic drugs through toxicity studies. For all the above reasons the topic 'Toxicological study of *Vatagajendra sinha Rasa* by L.D.50 method' has been studied.

AIM: *To study toxicological effects of "Vatagajendrasinha Rasa" in albino mice.*

OBJECTIVES

1) To determine lethal dose 50% (L.D.50) of "*Vatagajendrasinha* Rasa" in albino mice.
2) To study acute oral toxicological effects of "*Vatgajendrasinha Rasa"* in albino mice.

The material and methods include the **Following steps**

A. Selection of samples- was done by randomisation lottery method.
B. Standardization.
1. Qualitative Analysis - Confirmatory Tests showed the presence of cations like Mercury, Lead, Iron and Copper and anion like Sulphur by chemical test.
2. Quantitative Analysis- the following tests of Vata gajendra Sinha Rasa were carried out-
   a) Determination of Loss on drying
   b) Determination of total ash value
   c) Determination Acid insoluble ash
3. Heavy metal Analysis was also done.

C. Acute oral toxicity study Permission of (**CPCSEA)** committee for the purpose of control and supervision on animal of (**IAEC**) internal animal ethics committee of the Pharmacy College was taken prior to starting of experiment.

Acute oral toxicity study was carried out according to OECD 423 guidelines.3 female Albino mice were used for each dose testing.

- Average wt. of mice- 25-35 gm
- Age- between 8-12 weeks

- Grouping-mice were marked with picric acid for easy identification.

No of groups

1) Group A- Control group
2) Group B-300mg/kg
3) Group C-2000mg/kg
4) Group D-5000mg/kg

The weighed quantity of Sample was suspended by thorough mixing with 0.5% CMC (Carboxy Methyl Cellulose).

3) Each mouse was weighted accurately before dosing.

4) Mice were marked for easy identification by picric acid on their body parts like neck, paw and tail and named as N, P and T respectively.

5) Dose was calculated based on their body weight. Starting dose was decided as 300mg/kg.

6) Dose to be given was administered orally with the help of oral feeding needle.

7) Mice were observed individually after dosing continuously during first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter for 14 Days.

8) Mice were observed for 14 Days for following signs and symptoms:

* Behavioural changes
* Salivation
* Diarrhoea
* Urination
* Convulsions
* Coma
* Death

9) Weight was taken once in a week.

10) All the observations were noted and put in tabular form.

11) Limit dose was decided as 5000mg/kg and study terminated at this Stage

Observations

Observation of Qualitative Analysis and Heavy metal Analysis

Sr.	Parameters	Results	Units	Test method
1	Loss on drying	6.14	g/100g	Ranganna
2	Total Ash	33.01	g/100g	Ranganna
3	Acid Insoluble Ash	7.14	g/100g	Ranganna
4	Lead	0.11	g/100g	AOAC 972.25
5	Copper	0.17	g/100g	AOAC 999.11
6	Iron	0.066	g/100g	AOAC944.02,32.01.09
7	Mercury	0.0062	g/100g	AOAC 971.21

AOAC-Association of Official Analytical Chemist

Observation on acute oral toxicity study: Sample A- Control group

Signs and symptoms	Days	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
1. Weight	**N**	23.2						25.8							29.0
	P	22.8						25.4							29.0
	T	24.5						27.2							30.4
2.Behavorial changes	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	P														
	T														
3.Salivation	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	P														
	T														

4. Diarrhoea	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
5. Urination	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
6. Convulsions	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
7.Coma	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
8.Death	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N

All the three mice remained healthy for all fourteen days and showed no signs and symptoms of toxicity. Starting dose was decided as 300mg/kg.

Sample B -300mg/kg -observations from Day 1 – 14

Signs and symptoms	Days	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
1. Weight	N	22.3	-	-	-	-	-	25.1	-	-	-	-	-	-	28
	P	22.8						25.4							28.6
	T	24.9						27.6							31.2
2.Behavorial changes	N	A													
	P	A	N	N	N	N	N	N	N	N	N	N	N	N	N
	T	A													
3.Salivation	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4. Diarrhoea	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5. Urination	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6. Convulsions	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7. Coma	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8.Death	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N	N

A= anxiety **N=** no any abnormality noted.
All the three mice remained healthy for all fourteen days.

All three mice showed anxiety on day 1 but were not significant.

Sample C -2000mg/kg -observations from Day 1 – 14

Signs and symptoms	Days	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
1. Weight	**N**	23.4						26.3							29.2
	P	22.8						25.4							28.4
	T	24.3						27.2							30.5
2.Behavorial changes	**N**	A	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	P	A													
	T	A													
3.Salivation	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	P														
	T														
4. Diarrhoea	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	P														
	T														
5. Urination	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	P														
	T														
6. ConvulsionsP	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	T														
7. Coma	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	P														
	T														
8.Death	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	P														
	T														

A=anxiety
N=No any abnormality found.
All three mice showed anxiety on day 1 but were not significant.

Sample D -5000mg/kg -observations from Day 1 – 14

Signs and symptoms	Days	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
1. Weight	**N**	24.8						27.2							30.7
	P	22.4	-	-	-	-	-	25.6	-	-	-	-	-	-	28.1
	T	23.7						26.2							29.8
2.Behavorial changes	**N**	A		D	**N**		**N**	**N**	**N**	**N**		**N**	**N**	**N**	**N**
	P	A				D									
	T	A									D				

3.Salivation	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
4. Diarrhoea	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
5. Urination	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
6. Convulsions	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
7. Coma	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N
8.Death	N P T	N	N	N	N	N	N	N	N	N	N	N	N	N

A=anxiety

D=drowsy

N=No any abnormality found

All three mice showed anxiety on Day 1 but were not significant.

Mouse N showed drowsiness on Day 3 more than the other Days

Mouse P showed drowsiness on Day 5 more than the other Days

Mouse T showed drowsiness on Day 10 more than the other Days.

But this drowsiness was not much significant.

Autopsy

- The mice were anaesthetised with anaesthetic ether and organs were sent for histopathological examination.
- At dose of 300 mg/kg, 2000mg/kg and 5000mg/kg – liver and kidney appeared to be normal externally.

Histopathological examination

Group A Control group	Glomerulopathiae	Degeneration of tubules	cellular infiltration
P	-	-	-
T	-	-	-
N	-	-	-
GroupB P	-	-	-
T	-	-	-
N	-	-	-
Group C P	-	-	-
T	-	-	-
N	-	-	-
Group D			

P	+	+	+
T	++	++	++
N	+	+	+

Note

-: no abnormality detected

+: damage/ active changes up to less than 25 %

++: damage/ active changes up to less than 50 %

+++: damage/ active changes up to less 75 %

++++: damage/ active changes up to more than 75 %

Liver

Group A Control Group.	**swelling of hepatic cords**	**degenerative chang-es**	**cellular infiltration**
P	-	-	-
T	-	-	-
N	-	-	-
Group B 300 mg/kg P	-	-	-
T	-	-	-
N	-	-	-
Group C 2000 mg/kg P	-	-	-
T	-	-	-
N	-	-	-
Group D 5000 mg/kg P	+	+	+
T	+	+	+
N	+	+	+

Statistical Analysis

Group	Sex	Animal no.	Weight (in gm)recorded on day 0^{th}	7^{th}	14^{th}	Total weight gain
A	Female	01(N)	23.2	25.8	29.0	5.8
		02(P)	22.8	25.4	29.0	6.2
		03(T)	24.5	27.2	30.4	5.9
B	Female	01(N)	22.3	25.1	28	5.7
		02(p)	22.8	25.4	28.6	5.8
		03(T)	24.9	27.6	31.2	6.3
C	Female	01(N)	23.4	26.3	29.2	5.8
		02(P)	22.8	25.4	28.4	5.6
		03(T)	24.3	27.2	30.5	6.2

D	Female	01(N) 02(P) 03(T)	24.8 22.4 23.7	27.2 25.6 26.2	30.7 28.1 29.8	5.9 5.7 6.1

Statistical Analysis of Body Weight Changes:

Statistics analyzed by student's 't' test

Paired 't' test - To determine the significance in the body weight gain in all the groups before the dosing and after 14 days of dosing The level of significance is set at 5% or 0.05.

Unpaired 't' test - To compare the effect on the body weight gain in control group and test groups.

Groups	Mean observed difference	S.D.	S.E.	't' Value	P	Result
A Vehicle control)	5.97	0.20	0.11	54.27	<0.05	Significant
B (300mg/ kg)	5.94	0.32	0.18	33.00	<0.05	Significant
C 2000mg/kg)	5.87	0.30	0.17	34.52	<0.05	Significant
D(5000 g/kg)	5.90	0.20	0.11	53.63	<0.05	Significant

Result: The above table reveals that, there is significant body weight gain in all the groups before and after the dosing.

Unpaired test

Groups	Difference of mean	S.D.	S.E.	T value	P	Result
A & B	0.03	0.26	0.21	0.14	>0.05	Insignificant
A & C	0.10	0.25	0.20	0.50	>0.05	Insignificant
A & D	0.07	0.20	0.16	0.43	>0.05	Insignificant

Result: There is no difference or insignificant difference in the body weight gains in all the test groups when compared with vehicle control group i.e. there is no harmful effect on body weight gain in the test groups when compared with that of vehicle control group.

DISCUSSION

Market prepared sample was selected over self prepared sample as when we intentionally prepare a sample, we would take care of doing all the steps involved in its preparation in the best possible way .The purpose of this study is to check on an average, the safety of the drug. When we prepare the drug with all possible precautions it would be safe for sure and hence the purpose of study won't be served well.

Only one pharmacy drug was taken for the experiment as the sole purpose of the experiment was only to check its safety and not to compare between two pharmacies. No signs and symptoms of toxicity were seen even at300 mg/kg & 2000mg/kg but at the dose of 5000mg/kg little drowsiness was seen.

- **Body weight changes-**

Statistical analysis showed

1. Significant body weight gain in all the groups before and after the dosing

2. Insignificant difference in the body weight gains in all the test groups when compared with vehicle control group

This shows there is no harmful effect on body weight gain in the test groups when compared with that of vehicle control group.

CONCLUSION

1. Vatagajendra sinha Rasa is safe at 5000mg/kg as the LD 50 of Vatagajendra sinha Rasa > than 5000 mg/kg inspite of many metallic contents which may be due to the processes like shodhan & marana
2. Histopathological examinations reveal normal results at 300 and 2000mg/kg. At dose of 5000 mg/kg histopathology reveals some changes.
3. Hence it can be said that for Vatagajendra sinha Rasa the daily dose should be less than 5000 mg/kg & daily regimine should be decided with regular check on LFT, KFT. The clinical signs & symptoms should be co –related as possible.

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