ISSN 2456-3110
Vol 4 · Issue 4
July-Aug 2019

Journal of Ayurveda and Integrated Medical Sciences

www.jaims.in

JAIMS

An International Journal for Researches in Ayurveda and Allied Sciences

Charaka Publications

Indexed

Journal of
Ayurveda and Integrated Medical Sciences
ISSN 2456-3110
ORIGINAL ARTICLE July-Aug 2019

An experimental study to evaluate the concept of *Trividha Atisevana Varjya Dravya* w.s.r. to *Lavana*

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ABSTRACT

Background: *Lavana is* used as medicine as well as *Ahara* since ancient times. In *Caraka Samhita* it has been mentioned that three *Dravyas* viz. *Pippali*, *Kshara* (alkali) and *Lavana* (salt) can be used as emergency medicine, but they should not be consumed in excess (*Ati Upayunjita*). Hence in the present study *Lavana* has been evaluated in experimental animals in two different phases' viz. Acute administration at graded doses as part of acute toxicity study and Sub-Acute administration at fixed dose level, as part of toxic Sub-Acute toxicity study, to assess the possible adverse effects. **Materials & Methods:** Wistar strain albino rats of either sex weighing between 150 - 200g. body weights were used, The experiment was carried out in accordance with the direction of the Institutional animal ethics committee (IAEC) after obtaining its permission (Approval number IAEC – 138/k/2018). **Results:** Results were drawn based on histopathological reports and biochemical reports of each group of toxicity study. Acute toxicity study has been carried out in albino rats receiving the 2 dose level maximum at up to 10 times higher (855mg/kg) then the therapeutic equivalent dose (427.5mg/kg). In Sub-Chronic toxicity: dose given was five times higher than therapeutic equivalent dose and ten times the equivalent to human therapeutic dose for duration of 30 days. **Discussion:** Toxicity is not found in Acute study and in Sub-Acute study moderate to high toxicity is found.

Key words: *Acute, Sub-acute, Toxicity, Lavana, Salt.*

INTRODUCTION

Today's fast moving lifestyle and modernization has caused change in food pattern of human beings, and they have little time to think what they are eating is a healthy diet. Globalization has seriously affected one's eating habits and enforced many people to consume fancy and high calorie fast foods, popularly known as Junk foods. A set of fairly satisfactory dietic codes had been identified and prescribed by Ayurveda.

Any modifications in diets and even in their preparation style leads to ill health. Ayurveda explains the concept of *Nitya Sevaniya* (consumable) and *Atisevaniya Varjya Dravyas* (non conumable).

Trividha Atisevana Varjya Dravyas has been explained by *Acharya* Charaka in *Rasavimaniya Adhyaya* of *Vimanasthana*.

Pippali (Piper longum), *Kshara* (Alkali) and *Lavana* (salt) should not be consumed in excess quantity. If consumed it will cause various hazards. Among these three *Dravyas*, *Lavana* has been selected for the present study.

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Submission Date: 24/06/2019 Accepted Date: 16/08/2019

Access this article online

Quick Response Code

Website: www.jaims.in

DOI: 10.21760/jaims.4.4.26

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OBJECTIVE OF THE STUDY

1. To collect, compile and experimentally analyze the concept of '*Trividha Ati Sevaniya Varjya Dravya's* w.s.r. to Lavana.
2. To evaluate the effect of *Lavana (Samudra Lavana)* - experimentally in context of *Ati Sevana Varjya*.
3. To evaluate the sub-acute toxic effect of *Lavana (Samudra Lavana)* in albino rat two different dose levels.

MATERIALS AND METHODS

Materials

Test Drug

The test drug i.e. *Lavana* (sodium chloride) for the present study was procured from the Pharmacy of Rasashastra and Bhaishajya Kalpana, BLDEA'S AVS Ayurveda Mahavidyalaya, Vijayapur.

Experimental animals

- Wister strain albino rats were selected from Sri Venkateshwara Enterprises, suppliers of Laboratory animals, Bangalore. (CPCSEA =237).
- The rats were maintained under strict laboratory conditions, controlled with environmental temperature, humidity and light dark cycles.
- The rats were fed with balanced pellet diet as prescribed by CFTRI and water and libitum.

Inclusive criteria

- Healthy albino rats of either sex will be considered.
- Weighing about 150-200g
- Albino rats between 90 -120 days were included.

Exclusion criteria

- Rats less than 150g and more than 200g.
- Pregnant and diseased rats.
- Rats which are under trial of other experiments.

Mode of Administration

Drugs were administered through Oral route. 16 no. needle bent slightly at its tip and inserted into a cut IV tube to the length of needle to prevent oral injury. Needle fixed to the 2 ml syringe.

Plan of the Study

The study is planned in two different phases as follows,

- Acute toxicity study - 9 Albino rats
- Sub -acute toxicity study - 12 Albino rats

Method

The Toxicological studies will be conducted according to Ayush Guidelines for screening for toxicity in the Animal house attached to Dept. of Rasashastra and Bhaishajya. BLDEA'S AVS Vijayapur. Standard pharmacological protocol will be adopted for the experimental study which will be mentioned in the description of experimental study. Whole work is planned to generate data from laboratory i.e., experiments on animals are performed as described in references.

Rat dose was calculated on the basis of Human dose by using Standard Conversion Method on the basis of body surface area ratio using the table of Paget and Barnes.

Dose calculation

Recommended human dose of *Lavana* (Common Salt) - 5gms/day

Rat dose = Human dose x 0.018 (for 200gm of body weight)

= 5 x 0.018

= 0.09gm

= 90mg

Acute study

- Study was divided in to 3 groups. Each group contain 3 rats.
- Recommended test dose is given to all the groups.

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- Observed for 24 hrs, and after 24 hrs. Then scarifies was done. Blood is collected for laboratory investigation and organs sent for Histopathology.

No of Rats	Control Group	TED X 5	TED X 10
Rat 1	Water	382.5 mg	765 mg
Rat 2	Water	427.5 mg	855 mg
Rat 3	Water	427.5 mg	855 mg

Sub-acute Study

1. Study is divided in to 4 groups. Each group contain 3 rats.
2. Recommended dose is given to all the groups.
3. For 30 days dose is given. On 31st day scarifies is done.

No of Rats	Control Group	TED	TED X 5	TED X 10
Rat 1	Water	67.5 mg	382.5 mg	720 mg
Rat 2	Water	67.5 mg	427 mg	697.5 mg
Rat 3	**Water**	**67.5 mg**	**427 mg**	**697 mg**
TED - Therapeutic Equivalent Dose.				

Statistical analysis

The data generated is mentioned as Mean ± SEM. Difference among the groups is assessed by employing one way ANOVA with Dunnet's multiple't' test.

If P value is less than 0.05 - There is a significant difference.

If P value is more than 0.05 - There is no significant difference.

Observations and Results

Acute Group

No major difference has been observed in acute groups after giving respective doses. Even up to 855mg/kg dose level no mortality and behavioural changes were observed.

Sub Acute Group

- 3 rats in a group 4 TED X 10, 3 rats died during the experiment. 1st expired on 5th day during the experiment 2nd rat on 8th day, and 3rd rat on 9th day during experiment.
- The rats belonging to TED X 5 group, were looking unhealthy at the end of the experiment.

Acute Study

Effect on Haematological and Biochemical Parameters.

SN	Parameter	Control Group	TED X 5	TED X 10
1.	Total WBC count	NSD	NSD	NSD
2.	Diff. count	NSD	NSD	NSD
3.	Lymphocytes %	NSD	NSD	NSD
4.	Eosinophils %	NSD	NSD	NSD
5.	Monocytes %	NSD	NSD	NSD
6.	Basophils %	SD	SD	SD
7.	RBC count	NSD	NSD	NSD
8.	Haemoglobin %	NSD	NSD	NSD
9.	Heamacrite	NSD	NSD	NSD
10.	MCV	NSD	NSD	NSD
11.	MCH	NSD	NSD	NSD
12.	MCHC	NSD	NSD	NSD
13.	RDW	NSD	NSD	NSD
14.	Platelet count	SD	SD	SD

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15.	Platelet volume	NSD	NSD	NSD
16.	PCT	NSD	NSD	NSD
17.	Platelet distribution width	NSD	NSD	NSD
18.	SGOT	NSD	NSD	NSD
19.	SGPT	SD	SD	SD
20.	Blood urea	SD	SD	SD
21.	Alkaline phosphate	NSD	NSD	NSD
22.	S. creatinine	SD	SD	SD

NSD : Non Significant Difference, SD : Significant Difference

Table 4: Summary of the data on Histopathological changes of the organs recorded during acute toxicity study.

SN	Organs	Control Group	TED X 5	TED X 10
1.	Brain	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - NSC Rat 3 - SC	Rat 1 - NSC Rat 2 - SC Rat 3 - SC
2.	Left kidney	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC
3.	Right kidney	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC
4.	Left lung	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - SC Rat 3 - SC	Rat 1 -NSC Rat 2 -SC Rat 3 - SC
5.	Right lung	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - SC Rat 3 - SC	Rat 1 - NSC Rat 2 - SC Rat 3 - SC
6.	Liver	Rat 1 - NSC	Rat 1 - NSC	Rat 1 - NSC
		Rat 2 - NSC Rat 3 - NSC	Rat 2 - SC Rat 3 - SC	Rat 2 - NSC Rat 3 - SC
7.	Spleen	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC
8.	Heart	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - NSC Rat 3 - SC	Rat 1 - NSC Rat 2 - NSC Rat 3 - SC

NSC - No Significant Changes, SC - Significant Change.

Sub-acute Study

Effect on Haematological and Biochemical Parameters

SN	Parameter	Control Group	TED	TED X 5
1.	Total WBC count	NSD	NSD	NSD
2.	Diff. count	NSD	NSD	NSD
3.	Lymphocytes %	NSD	NSD	NSD
4.	Eosinophils %	NSD	NSD	NSD
5.	Monocytes %	NSD	NSD	NSD
6.	Basophils %	NSD	NSD	NSD
7.	RBC count	NSD	NSD	NSD
8.	Haemoglobin %	NSD	NSD	NSD
9.	Heamacrite	NSD	NSD	NSD
10.	MCV	NSD	NSD	NSD
11.	MCH	NSD	NSD	NSD
12.	MCHC	NSD	NSD	NSD
13.	RDW	NSD	NSD	NSD

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14.	Platelet count	NSD	NSD	NSD
15.	Platelet volume	NSD	NSD	NSD
16.	PCT	NSD	NSD	NSD
17.	Platelet distribution width	NSD	NSD	NSD
18.	SGOT	NSD	NSD	NSD
19.	SGPT	NSD	NSD	NSD
20.	Blood urea	NSD	NSD	NSD
21.	Alkaline phosphate	SD	SD	SD
22.	S. creatinine	SD	SD	SD

Summary of the data on Histopathological changes of the organs recorded during sub-acute toxicity study.

SN	Organs	Control Group	TED	TED X 5	TED X 10
1.	Brain	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - SC	Rat 1 - NSC
2.	Left kidney	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC
3.	Right kidney	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 -SC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC
4.	Left lung	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - SC Rat 3 - SC	Rat 1 - SC Rat 2 - SC Rat 3 - SC	Rat 1 - SC
5.	Right lung	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - SC Rat 3 - SC	Rat 1 - SC Rat 2 - SC Rat 3 - SC	Rat 1 - SC
6.	Liver	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - NSC Rat 3 - SC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC
7.	Spleen	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	
8.	Heart	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - SC Rat 2 - NSC Rat 3 - SC	Rat 1 - NSC Rat 2 - NSC Rat 3 - NSC	Rat 1 - NSC

DISCUSSION

In the present study *Lavana* is taken, there are five types of *Lavana*, in which *Samudra Lavana* is used for present study, among the types of *Lavana*'s, *Saindhava Lavana* is said to best for internal use.

Acute Study

There are totally 22 parameters referred in blood investigation report. Statistically we see significant difference in Basophils and mild increase in TED X 5 group in Rat 2, may be due to chronic non-specific inflammation in some organs. Statistically there is no

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significant difference in all RBC related parameters. All are normal. Statistically there is significant difference, we find mild increase in platelet count in TED X 5, TED X 10 group Rats due to chronic nonspecific inflammation in some organs. Statistically we see that there is a significant difference in SGPT, Blood urea, S. Creatinine and moderately increase in SGOT levels in TED X 5 and TED X 10 groups in all rats, increase in SGPT in TED X 10 group rats, and increase in Alkaline phosphate in TED X 5, TED X 10 group rats, these all may be due to Chronic Nonspecific Inflammation. Over all there is a mild to moderate increase in some parameters in blood.

In the present study, in acute there are 3 groups, In Group 1 (control group) histopathology level all the organs are normal, in this group experimental drug was not given to rats only water was given to rats. Histopathology report of group 3 (TED X 5) and group 4 (TED X 10). In present Acute study, in group 2 (TED X 5) Rats, histopathologically we found that in both the lungs of all 3 Rats there was a congested blood vessels and diffuse lymphocytic infiltration to interstium and occasional lymphoid aggregates were noted, due to this there is a mild increase in Basophill count and mild increase in platelet count in all three Rats. There is a mild increase in SGOT and SGPT and alkaline phosphate in Rat 2 and Rat 3, this may be due to congested blood vessels and mononuclear cell infiltration the portal triad also noted fatty changes at the places in liver. All these suggest that least amount of toxicity was found in acute study of group 2. In Group 3 (TED X 10) , in Rat 2 and Rat 3, we found congested blood vessels and occasional perivascular lymphocytic infiltration in brain and focal area of haemorrhage, this may also occur in process of death. And in Rat 3 in liver, histopathologically we found congested blood vessels and mono nuclear cell infiltration portal triad and also noted fatty changes at places due to this there is a moderate increase in SGOT, SGPT and alkaline phosphate. There is a mild change in platelet count may be due to congested blood vessels and diffuse lymphocytic infiltration in to interstium, occasional lymphoid aggregates seen in right and left lung in Rat 2 and Rat 3. All these suggest that there might be a mild toxic level in acute group 3 at dose level of TED X 10.

Sub Acute Study

Statistically there is no significant in all rats. In group 2 there is mild increase in WBC count in rat 3, and in group 3,mild increase in WBC count in rat 1,2,3 and mild increase in lymphocytes in all rats of group 2 and group 3. May be due to chronic nonspecific inflammation in some organs. Other parameters are normal. Statistically there is no significant difference in all RBC related parameters. May be due to chronic non-specific inflammation in some organs. Other parameters are normal. Statistically there is no significant in all rats. But mild increase in platelet count in group 2 and group 3 in rat 2 &3. May be due to chronic non-specific inflammation in some organs. Other parameters are normal. There is statistically significant difference in Alkaline phosphate and S. Creatinine in all groups. In group 2 there is mild increase SGOT,SGPT, moderate increase in alkaline phosphate in rat 3 this may be due to chronic nonspecific inflammation where as in group 3 mild increase in alkaline phosphate in rat 1, rat 2 , highly increase in SGOT, SGPT in rat 1 and in rat 3 may be due to chronic non-specific inflammation.

In Histopathalogy study, in sub-acute group 2 (TED), histopathalogy in both lungs of Rat 1 and Rat 2 found congested blood vessels were seen diffused lymphocytic infiltration in to interstium and a place of lymphoid aggregates were seen, this causes mild variation in WBC count and mild increase in platelet count. Left lung is normal in Rat 3 where as in right lung congested blood vessels and focal area of haemorrhage was seen. Mild increase in SGOT, SGPT, moderate increase in alkaline phosphate was seen, this may be due to congested blood vessels and focal area of haemorrhage in Rat 3 in right kidney. This suggests that there may be mild toxic effect in all Rats.

In Group 3 - in Rat 3, there is a mild increase in WBC count and platelet count which may be due to congested blood vessels and focal areas of haemorrhage and mono nuclear cell Infiltration in right and left lung in Rat 2 & 3, there is a congested

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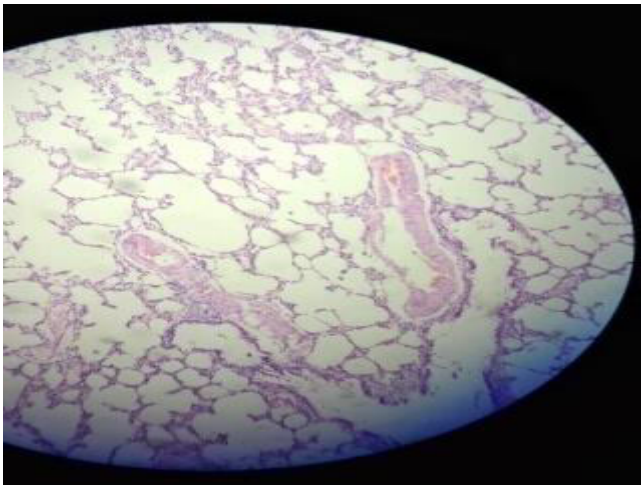
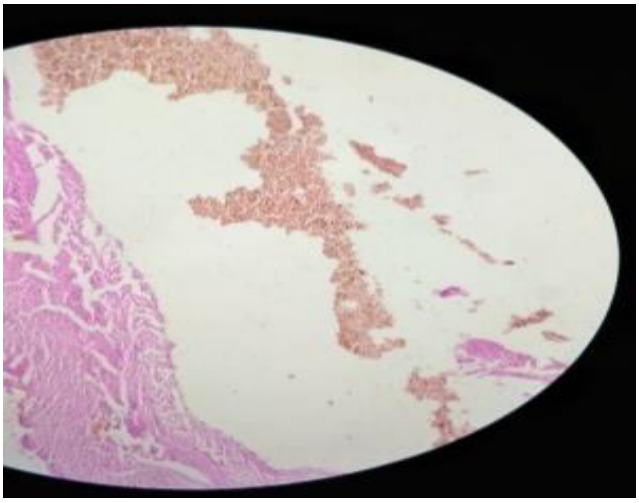
blood vessels were also seen, diffused lymphocytic infiltration in to interstium at places, lymphoid aggregates were seen, this causes highly increase in SGOT & mild increase of Alkaline phosphate in Rat 1 & 3. All these suggest that moderate toxicity can be found in group 3 (TED X 5). As discussed earlier all Rats died in between the experiment, only one Rat organs were able to send for histopathology study. Report says that there is a congested blood vessels and lymphocytic infiltration in portal triad. At this dose level TED X 10 toxicity is found. All these suggests that Highly toxicity can be seen in group 4 (TED X 10).

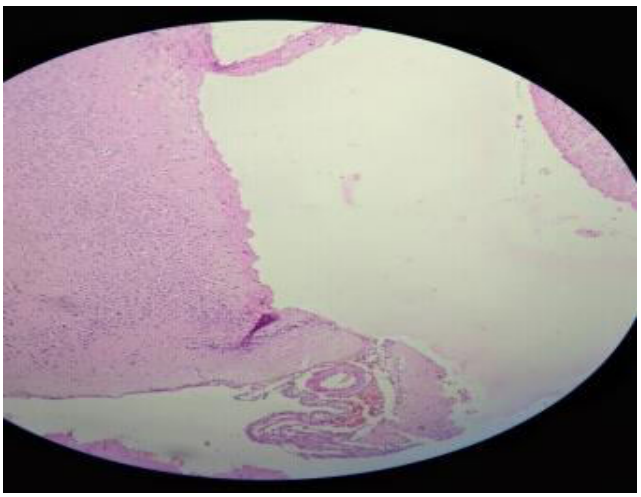
Microphotographs Acute toxicity Study (Ted X 10)



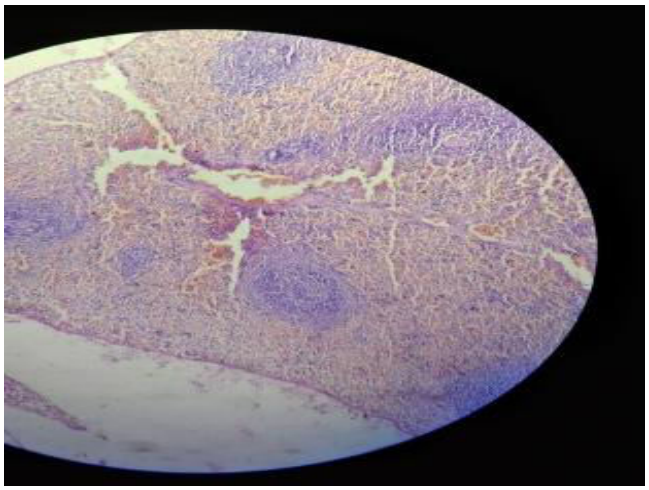
Left Kidney Normal



Heart Normal



Brain Occasional Lymphostic Infilteration



Spleen Normal

CONCLUSION

Based on present study, *Lavana* is again proved to be *Atisevaniya Varjya Dravya*. So we should not consume excess to avoid health hazards. By animal experiment, we found in acute group no toxicity was found, where as in Sub-acute group moderate to high toxicity was found. Thus *Lavana* should not be consumed in excess.

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How to cite this article: Dr. Shilpa Nimbal, Dr. Umapati C. Baragi, Dr. Kashinath Hadimur, Dr. Jyothi Alias Jyostna. An experimental study to evaluate the concept of Trividha Atisevana Varjya Dravya w.s.r. to Lavana. J Ayurveda Integr Med Sci 2019;4:191-198. http://dx.doi.org/10.21760/jaims.4.4.26

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

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