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 experiemental study to evaluate the principle *Trini Dravyani Nati Upayunjita* w.s.r. to *Kshara* (Alkali)

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ABSTRACT

Background: In *Charaka Samhita* it has been mentioned that three medicinal substances viz. *Pippali* (*Piper longum*), *Kshara* (alkali) and *Lavana* (salt) can be used as emergency medicine, but they should not be consumed in excess (*Ati Upayunjita*). If they are consumed in excess quantity they will cause several adverse effects in the body. Hence in the present study *Kshara* 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 sub-acute toxicity study, to assess the possible adverse effects if any. **Objectives:** To evaluate the acute and sub-acute toxic effect of *Kshara* in albino rats to establish the principle of *Trini Dravyani Nati Upayunjita.* **Materials and Methods:** Wister strain albino rats of either sex weighing between 150 - 200g body weights were used for experimental study. The experiment was carried out as per 'Ayush Guidelines' after the IAEC clearance. For Acute Toxicity - 9 Albino rats were used and for Sub-Acute Toxicity - 12 Albino rats were used. The dose calculation was done on the basis of body surface area ratio using the table of 'Paget and Barnes rule'. **Results:** In Acute toxicity study no mortality and behavioral changes were observed when the drug *Kshara* was studied after two dose level i.e. TED X 5 and TED X 10. In Sub-acute study some behavioral changes (including cage side behavior) were observed. No mortality was observed in any of the groups. **Discussion:** Acute toxicity study of *Kshara* showed no immediate and evident toxic signs and mortality within 24 hours of observation. In Sub-acute toxicity study in all four groups, no mortality or evident toxic effects were observed, however some mild histopathological changes were observed in sub-acute study.

Key words: *Kshara, Alkali, Sodium bicarbonate Acute toxicity study, Sub-acute toxicity study.*

INTRODUCTION

Today's fast moving world has been adapted to a system of consuming of foods which has several adverse effects on human wellbeing. Lifestyle modifications and change in food pattern has compelled us so much that one has so little time to really 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.[1]

There is an old saying that, "We are what we eat" which holds good even today. Ailments like Obesity, food poisoning, dehydration, cardiac problems, diabetes mellitus and arthritis have seen a profound rise in developing countries and such unhealthy junk food, processed food, packed food, high fat calorie consumption are the notable factors to its contribution.[2]

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Submission Date: 25/06/2019 Accepted Date: 19/08/2019

Access this article online

Quick Response Code

Website: www.jaims.in

DOI: 10.21760/jaims.4.4.24

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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. Various pathological entities stand identified as a result of food habits related as they are to sensory stimuli, which are pleasure giving, or distress giving. Even though Ayurveda has postulated this theory and has dealt in details, only recently a great deal of interest has been focused on the art of dietary factors in the pathogenesis of non-communicable diseases. And there are volumes of scientific data supporting the theory that diet is the underlying key factor in most chronic degenerative disorders and heart ailments.

Ayurveda explains the concept of *Nitya Sevaniya* (daily consumable diet) and *Atisevaniya Varjya Dravyas* (non consumable diet), *Acharya Charaka* has also explained that *Pippali* (Piper longum), *Kshara* (Alkali) and *Lavana* (salt) should not be consumed in excess quantity. If consumed it will lead to various hazards. Among these three *Dravyas*, *Kshara* (Alkali) has been selected for the present experiemental study.

Acharya Charaka has told that its excess use produces injurious effects on hair, eyes, heart, virility and people may suffer from blindness, impotency, baldness, grey hair and various heart diseases.

Background for selection of *Kshara* (Sodium Bicarbobate) for its toxicity evaluation

Kshara i.e. Sodium bicarbonate, referred to as baking soda / baking powder, is fairly used in baking of pancakes, cakes, quick breads, soda bread, and other baked food products. Food preparations like Papad, Pickles, Cold drinks and Eno etc. which are commonly consumed has chief ingredient as sodium bicarbonate.

Kshara has been used as medicine as well as food since ancient times. In Caraka Samhita it has been mentioned that three medicinal substances viz. *Pippali*, *Kshara* (alkali) and *Lavana* (salt) can be used as emergency medicine, but they should not be consumed in excess (*Ati Upayunjita*). If they are consumed in excess quantity they will cause several adverse effects in the body. There is no specific explanation available for the term *Ati Upayunjita*. However excess use also can be taken in two ways i.e. in high dose and/ or for continuous use for long duration. For the drug *Kshara*, the word *Ati Upayunjita* is mentioned for continuous use for longer duration.[3] What time period considered being a long time to use has not been clarified by the Acaryas. Hence in the present study *Kshara* was evaluated in experimental animals in two different phases viz. acute administration at graded doses as part of Acute toxicity study and chronic administration at fixed dose level, as part of Sub-acute toxicity study, to assess the possible adverse effects if any.

Materials and Methods

Animals

Wister strain albino rats of either sex weighing between 150 - 200g body weights were used for experimental study. The animals were obtained from Venkateshwara Enterprises, Bengaluru. The animals were exposed to natural day and night cycles under ideal laboratory conditions in term of ambient temperature (28+2°C) and humidity (50-60%). They were fed with Amrut brand rat pellet supplied by Pranav Agro Industries and tap water. The experiment was carried out as per 'Ayush Guidelines' of Ayurveda and Siddha plants drugs for that IAEC clearance was obtained. The experiment was carried out in accordance with the direction of "Institutional animal ethics committee (IAEC)" (Approval number IAEC; 533/GO/Re/S/02/CPCSEA).

Test drug

Test drug i.e. *Kshara* (Baking Soda / Sodium Bicarbonate / $NaHCO_3$) was used for this study. The test drug i.e. *Kshara* (Baking Soda) was authentified by the experts of Pharmacognist.

Sample

For Acute Toxicity - 9 Albino rats.

For Sub-Acute Toxicity - 12 Albino rats.

Inclusion criteria

1) Healthy Albino Rats of either sex were considered.

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2) Weighing about 150 - 200g.

Exclusion criteria

1) Rats less than 150g and more than 200g.
2) Pregnant and diseased rat.
3) Rats which were under trial of other experiments.

Dose fixation and schedule

The dose calculation was done on the basis of body surface area ratio using the table of "Paget and Barnes rule".

Calculation of dose[4]

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

Therapeutically Human dose of *Kshara* (Sodium bicarbonate) 1.250 gm/day

So, the suitable dose for rats was calculated by referring to table of 'Paget and Barnes rule'.

i.e. Rat dose = human dose X body surface area ratio convertibility factor

= 1250 X 0.018

= 22.5 mg / 200 gm body wt of rat.

Mode of Administration

Drug were administered through Oral route. 18 number needle bent slightly at its tip and inserted into a cut IV tube to the length of needle to prevent oral injury. Needle was fixed to the 2ml syringe.

Acute toxicity study

Acute Toxicity study was conducted according to 'AYUSH Guidelines',[5] Drug was given in 3 dose level. i.e. Control group, TED X 5, TED X 10, 9 Albino rats were used of either sex, Study was divided into 3 groups, each group contain into 3 rats, Recommended Test dose was given to all the groups, The solution was administered orally by using of syringe and needle. The solution should be given 1 ml, Observed for 24 hours, After 24 hours sacrifices was done, Then blood was collected and sent for laboratory and organs sent for histopathology.

Table 1 : Grouping and dose fixation for acute toxicity study

Group	No of Rats	Drugs	Rat No	Dose	Rat body wt. before experiment	Rat body wt. after experiment
I (Control group)	3	Water	R1	QS	156 gm	220 gm
			R2	QS	170 gm	200gm
			R3	QS	165 gm	215 gm
II (TED X 5)	3	Kshara	R1	99mg	175 gm	205 gm
			R2	90mg	160 gm	240 gm
			R3	105 mg	185 gm	190 gm
III (TED X 10)	3	Kshara	R1	214 mg	190 gm	202 gm
			R2	192 mg	170gm	198 gm
			R3	220 mg	195gm	195 gm
Frequency and duration of dose – once only						

Sub-acute toxicity study

Drug was given in 4 dose levels i.e. control group, TED (Therapeutic equivalent dose), TED X 5, TED X 10, 12 Albino rats were used of either sex, Study was divided into 4 groups, each group contains 3 rats, Recomended test dose was given to all groups, The solution was administered orally by using of syringe and needle. The solution was given in quantity of 1ml, For 30 days dose was given, After 31 days sacrifies was done, Blood sample was collected and organ was collected for further investication.

Table 2 : Grouping and dose fixation for sub-acute toxicity study

Group	No of Rats	Drugs	Rat No	Dose	Rat body wt. before experiment	Rat body wt. after experiment
I (Control group)	3	Water	R1	QS	160 gm	225 gm
			R2	QS	150 gm	205 gm
			R3	QS	150 gm	210 gm

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II (TED)	3	Kshara	R1	39mg	170 gm	204 gm
			R2	34mg	150 gm	204 gm
			R3	34mg	150 gm	195 gm
III (TED X 5)	3	Kshara	R1	203 mg	170 gm	205 gm
			R2	192 mg	179 gm	195 gm
			R3	192 mg	170 gm	200 gm
IV (TED X 10)	3	Kshara	R1	360 mg	160 gm	202 gm
			R2	383 mg	170 gm	198 gm
			R3	383 mg	170 gm	195 gm
Frequency and duration of dose – once only						

Parameters studied

- **Changes of body weight :** Changes of body weight was recoded on every 8 days.
- **Effect of food intake :** Quantity of food intake was recorded weekly.
- **Feacal consistency:** Feacal consistency changes was recorded weekly.
- **Heamatological parameters:** Complete blood count (Total WBC count, Neutrophils, Lymphocytes, Eosinophils, Monocytes, Basophils, Absolute Neutrophil Count, Absolute Lyphocyte Count). RBC Count (Hematocrit [PCV], MCV, MCH, MCHC, Red Cell Dist Width [RDW], Platelet Count, Mean Platelet Volume [MPV])
- **Biochemical Parameters:** Blood urea, Serum creatinine, SGOT, SGPT, Alkaline Phosphatese (ALP).
- **Histopathological Studies:** Histopathology of important organs like Heart, Lung, Liver, Spleen, Kidney, Brain has been carried out.

Statistical analysis

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

Observations and Results

Body weight

The body weight of albino rats before and after experiment among the three groups of acute toxicity study. It is observed that normal body weight gain in control group as well as test drug administration groups. The mean value of control group is 163.67 (SD=7.09), before experiment and 211.67 (SD=10.41), after experiment. Mean value was increased in control group of acute toxicity study. So, it is found statistically significant difference at P=0.0397.

The body weight of albino rats between the four groups i.e. group I (Control group), group II (TED), group III (TED X 5), group IV (TED X 10) of sub-acute toxicity study. It is observed that normal body weight increase in control group as well as test drug administration groups of all parameters. Before experiment observed that the mean value of weight in group I is 153 (SD=5.77, SE=3.33), group II is 156.670 (SD=11.55, SE=6.67), group III is 173.33 (SD=5.78, SE=3.33) and group IV is 166.67 (SD=5.78, SE=3.33). Mean value is increase in all groups of sub-acute toxicity study. So, it is found statistically significant difference at P=0.0432.

Food Intake

When compare intake of food in control group, TED X 5 and TED X 10 , after treatment in acute toxicity study. The mean value and SD are same and there is a significant difference at the P=0.0224. When compare intake of water in Control group, TED X 5 and TED X 10 after treatment in acute toxicity study. The mean value of all groups is comparatively increase. The mean value of control group is 18.33 (SD=3.15, SE=2.03),TED X 5 group is 23.67 (SD=1.16, SE=0.67) and TED X 10 group is 25 (SD=1.0, SE=0.58). So, it is found statistically significant difference at the P=0.0237.

Result - When compare intake of food in control group, TED, TED X 5 and TED X 10 in sub-acute toxicity study. In control group the mean value of food is

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18.23(SD=51.27, SE=0.73), in TED group the mean value is 18.76(SD=2.48, SE=1.43), in TED X 5 group the mean value is 19.91(SD=1.87, SE=1.08), and in TED X10 group the mean value is 21.53(SD=2.63, SE=1.37). So, there is found statistically no significant difference at the P=0.2819. When compare intake of water in control group, TED, TED X 5 and TED X 10 in sub-acute toxicity study. In control group the mean value of food is 30.8(SD=4.24, SE=2.45), in TED group the mean value is 30.37(SD=1.25, SE=0.72), in TED X 5 group the mean value is 32.45(SD=0.28, SE=0.16), and in TED X10 group the mean value is 33.34(SD=0.92, SE=0.53). So, there is found statistically no significant difference at the P=0.3893.

Hematological Parameters

The results obtained as regards hematological parameters throughout both acute and sub-acute toxicity study in albino rats have been provided in the form of consolidated statement in table no. 3 and 4.

Table 3: Effect on Hematological Parameters in acute toxicity study.

SN	Parameter	Control group	TED X 5	TED X 10
1.	WBC count	NS	NS	NS
2.	Neutrophils	NS	HS	HS
3.	Lymphocytes	NS	HS	HS
4.	Monocytes	NS	NS	NS
5.	Eisonophils	NS	HS	HS
6.	Basophils	NS	NS	NS
7.	RBC Count	NS	NS	NS
8.	MCH	NS	NS	NS
9.	MCHC	NS	HS	HS
10.	MCV	NS	NS	NS
12.	RDW	NS	HS	HS
13.	Hb	NS	NS	NS
14.	PCT	NS	HS	HS
15.	PCV	NS	NS	NS
16.	Platelet count	NS	HS	HS

NS - Non Significant, HS - Highly Significant

Table 4: Effect on Hematological Parameters in Sub-acute toxicity study.

SN	Parameter	Control group	TED	TED X 5	TED X 10
1.	WBC count	NS	NS	NS	NS
2.	Neutrophils	NS	NS	NS	NS
3.	Lymphocytes	NS	NS	NS	NS
4.	Monocytes	NS	NS	NS	NS
5.	Eisonophils	NS	NS	NS	NS
6.	Basophils	NS	HS	HS	HS
7.	RBC Count	NS	NS	NS	NS
8.	MCH	NS	NS	NS	NS
9.	MCHC	HS	HS	HS	HS
10.	MCV	NS	NS	NS	NS
12.	RDW	NS	NS	NS	NS
13.	Hb	NS	NS	NS	NS
14.	PCT	NS	HS	HS	HS
15.	PCV	NS	NS	NS	NS
16.	Platelet count	NS	HS	HS	HS

NS - Non Significant, HS - Highly Significant

WBC related parameters

In acute toxicity study, six parameters were studied, under WBC related parameters complete blood count observed that Neutrophils, Lymphocytes and

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Eisonophils were highly significant of all drug induced rats.

RBC related parameters

In acute toxicity study, ten parameters were studied, under RBC related parameter MCHC and RDW were highly significant of all drug induced rats and others non-significant and MCHC was highly significant of all drug induced rats and others non-significant in Sub-acute toxicity study.

Table 5: Effect on biochemical parameters of the serum in acute toxicity study.

SN	Parameter	Control Group	TED X 5	TED X 10
1.	Blood urea	NS	NS	NS
2.	S. creatinine	HS	HS	HS
3.	S.G.O.T.	NS	NS	NS
4.	S.G.P.T.	NS	NS	NS
5.	Al phosphatise	NS	NS	NS

Table 6: Effect on biochemical parameters of the serum in Sub-acute toxicity study.

SN	Parameter	Control group	TED	TED X 5	TED X 10
1.	Blood urea	NS	NS	NS	NS
2.	S. creatinine	HS	HS	HS	HS
3.	S.G.O.T.	NS	NS	NS	NS
4.	S.G.P.T.	NS	NS	NS	NS
5.	Al phosphatase	HS	HS	HS	HS

Total five biochemical parameters were studied. Test drug at the highest dose level did not change any of the biological parameters calculated to significant extent after 30 days of drug administration. Some parameters were changed, i.e. serum creatinine and alkaline phosphatase, S.G.O.T., S.G.P.T. in acute and sub-acute toxicity group.

Discussion on Histopathological parameter of different organs

Liver: In acute toxicity study microscopic examination of liver section showed congested blood vessels of the first rat, which is mild toxicity and others normal histopathology in TED X 10 group. Blood Congested was found of the third rat, which is non-specific and mild toxic effect and no specific histopathology noted of others rat in TED X 5 group.

In sub-acute toxicity study showed mild inflammation of first rat due to non-specific inflammation which is mild toxic effect and congested and dilated central vein, fatty changes and perivenular hemorrhagic necrosis of the second rat due to congestion and hemorrhagic necrosis, it was mild toxic effect in TED X 10 group. Congested blood vessel, fatty changes and mononuclear cell infiltration in the portal triad was found of the first and third rat due to chronic non-specific inflammation, which also mild toxic effect and normal histopathology was found of the others rat in TED X 5 group. All rat in TED groups, showed congested blood vessels and normal histopathology noted.

Lung: In acute toxicity study microscopic examination of right and left lung section showed congested blood vessels of first and second rat, which is mild toxic effect and also seen diffuse lymphocytic infiltration into interstium due to chronic non-specific inflammation, which is mild toxic effects in TED X 10 group.

In sub-acute toxicity study showed congested blood vessels, areas of hemorrhage and lymphocytic infiltration into interstium, at places lymphoid aggregates seen of all rats due to chronic non-specific inflammation in TED X 10 group and TED X 5 group and all rats of TED group showed congested blood vessels, areas of hemorrhage and lymphocytic infiltration due to chronic non-specific inflammation, which are also mild toxic effects.

Heart: In acute toxicity study microscopic examination of section of heart showed that congested blood vessels of first rat, which is mild toxic effect and others normal histopathology in TED X 10 group. No

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specific histopathology noted of all rats in TED X 5 group.

In sub-acute toxicity study showed congested vessels and focal areas of hemorrhage of all rats in TED X 10 group, which is mild toxic effect. Focal areas of hemorrhage noted of first rat, which is mild toxic effect and others normal histopathology noted in TED X 5 group. Congested blood vessels showed of all rats in TED group, which are non-specific.

Spleen: In acute toxicity study microscopic examination of section of spleen showed congested vessels in TED X 10 group. No specific histopathology noted of all rats in TED X 5 group.

In Sub-acute toxicity study showed congested blood vessels and areas of hemorrhage of all rats in TED X 10 group. No specific histopathology noted of all rats in TED X 5 group. Congested blood vessels and areas of hemorrhage noted of all rats in TED group, which are mild toxic effect.

Kidney: In acute toxicity study showed congested blood vessels of all rats in TED X 10 group. No specific histopathology was found of all rats in TED X 5 in the section of right and left kidney in acute toxicity study, but serum creatinine is increased due to chronic non-specific inflammation.

In sub-acute toxicity study showed congested vessels of the first rat and no specific histopathology noted of the second rat in TED and TED X 5 groups.

Brain: In acute toxicity study microscopic examination of brain showed focal ischemic necrosis of third rat of TED X 5 and TED X 10 group due to chronic nonspecific inflammation, In Sub-acute toxicity study in TED X 10 group of all rats showed congested blood vessels, which is mild toxic effect normal histopathology noted. The third rat of TED X 5 and TED X 10 groups showed focal ischemic necrosis congested blood vessels areas of hemorrhage and sparse lymphocytic infiltration noted of others rat due to chronic non-specific inflammation, which is mild toxic effect.

DISCUSSION

No mortality changes were observed. In this toxicity study no behavioural changes were observed, No exitus (death) was observed in all dose levels, During Acute toxicity studies the animals were observed for following sign and symptoms - general appearance, increased or decreased motor activity, anaesthetic, convulsion, lacrimation, salivation, diarrhoea, muscle spasm, arching and rolling, muscle relaxation, narcosis, irritability, tremours, straub reaction, CNS depression, analgesia, mode of respiration, changes in skin colour etc. But there was no changes observed in rats in three groups of Acute toxicity study.

Discussion on toxicity effect on albino rats

Out of six organs, changes were observed in Liver, Brain, Kidney and Lungs. In Liver, congested and dilated central vein was observed, fatty changes of both groups in lower dose levels and moderate inflammatory changes and necrosis was observed in highest dose levels. Considering the changes are in low magnitude, it can be suggested that the changes are not serious in nature.

In acute toxicity study

TED X 5 and TED X 10 Group

The present study of group II (TED X 5) and group III (TED X 10), complete blood count report says that, there is mild to moderate increase in total WBC count level and as well as lymphocytes level also increase and simultaneously slightly decrease in eosinophils count. Because of alteration in blood picture like hemotocrit value and mean corpuscular volume (MPV) also increasing, along with there is drastic changes of platelet count, there is mild to moderate increase of platelet count i.e. Pancytopenia and plateletcrit (PCT) level is also increase. Special investigation like LFT (Liver function test), KFT (Kidney function test) and serum electrolytes, LFT suggest that mild increase S.G.O.T and S.G.P.T. level of those rats. Whereas KFT giving a clear cut picture where increasing serum creatinine level of those rat.

In histopathological report right and left lung showed that, congested blood vessels and areas of hemorrhage and sparse lymphocytic infiltration into interalveolar septae due to chronic non-specific inflammation for any type of infection.

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Histopathology architecture of right and left kidney showed that, congestion of blood vessels because there is a mild dilation of glomerular and thickening of basement membrane space between glomerular membrane and bowmen's capsule is decrease, due to the tubules cell are seen of only increased. Overall architecture of kidney gives a clear cut picture of 'Acute nephritic syndrome' of change.

So, in acute toxicity group of all drug induced rats showing nephrotic syndrome changes the histopathology architecture which is well supported biochemical parameters altered KFT and LFT. It also showed that blood routine investigation and alteration.

Sub-acute toxicity study

TED, TED X 5 and TED X 10 Group

The present study of group I (TED) group II (TED X 5) and group III (TED X 10), compared biochemical report says that, serum creatinine level is increased. Increasing of serum creatinine level which disturbs Liver and LFT suggest that mild increase S.G.O.T, and S.G.P.T. level.

In histopathological report Liver showed that, congested blood vessels and mononuclear cell infiltration in the portal triad due to mild to moderate alter infiltration and also noted fatty changes which are supportive.

Due to increase of serum creatinine level which even after altered Cerebrospinal Fluid (CSF) composition and alteration the particular area i.e. focal lesion necrosis due to focal ischemia.

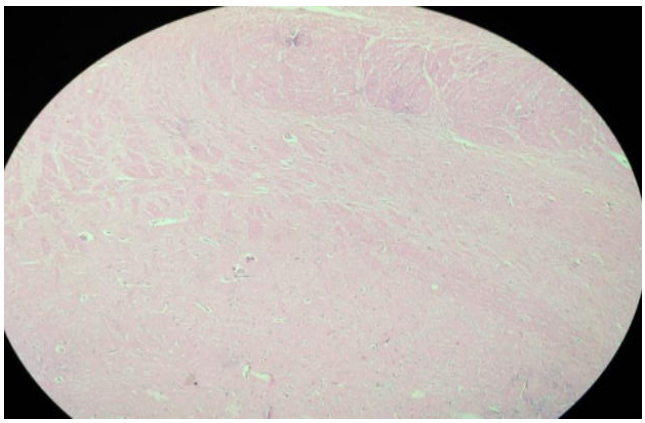
In histopathological report right and left lung showed that, congested blood vessels and areas of hemorrhage and sparse lymphocytic infiltration into interalveolar septae due to chronic non-specific inflammation for any type of infection.

So, in Sub-acute toxicity group of all drug induced rats showing focal necrosis of Brain due to ischemic condition. The histopathology architecture which is well supported biochemical parameters altered KFT and LFT and it also showed that blood routine investigation and alteration.

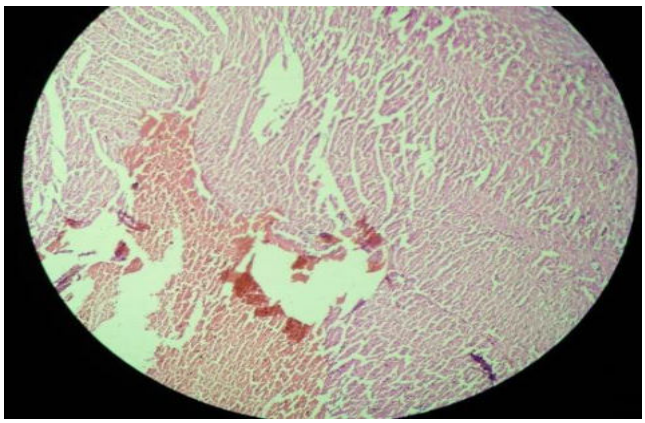
Literature review has shown that number of investigators have reported presence of range of pharmacological activities some with toxicological implications. It is necessary to discuss their relevance to possible toxicological effects occurrence in clinical trials.

No death occurs in whole study. So, thus overall analysis of the data generated during the present study clearly indicate that at the therapeutic dose level and even at the ten times of therapeutic dose level of *Kshara* even on long duration of administration do not have the potential to produced serious toxicity in persons who have no serious deficiency in functioning of the vital organs like liver, kidney, brain, lungs etc.

Microphotographs of acute toxicity study (TED X 10)



Brain Focal Ischemic Necrosis



Heart Normal

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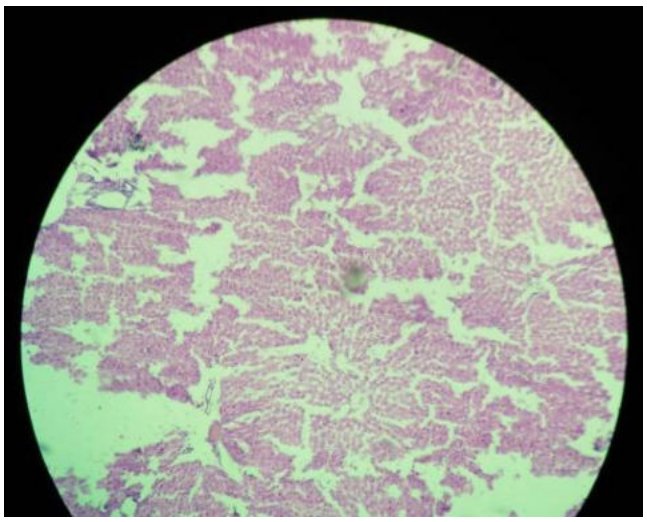
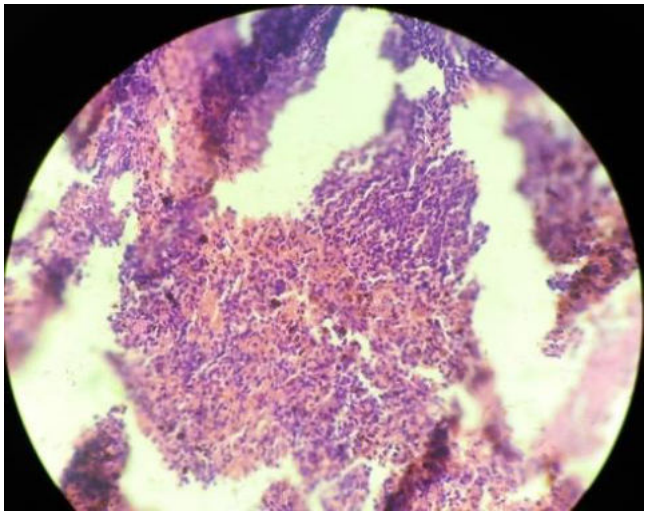
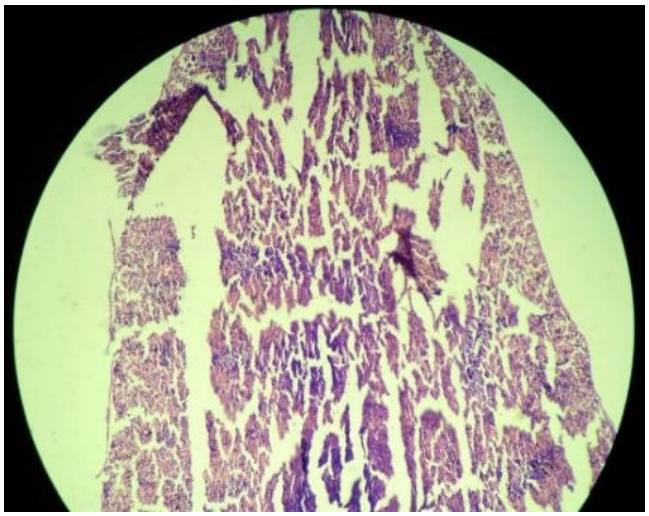


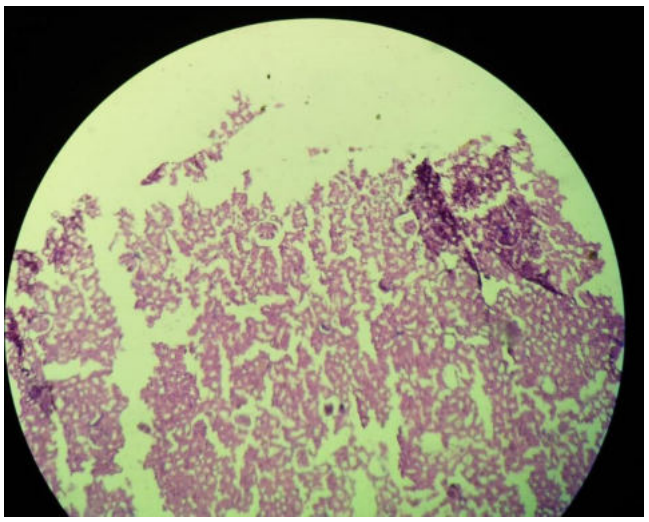
Liver Congestion



Spleen Congestion



Lung Congestion



Kidney Congestion

CONCLUSION

The present study was conducted to assess the toxicological effect of when *Kshara* in albino rats, when consumed in excess amount and for a longer duration in one or other way. In Acute toxicity study of *Kshara* (Baking Soda), no immediate and evident toxic signs and mortality was observed within 24 hours. In Sub-acute toxicity study of all four groups no mortality or evident toxic effects were observed. However the histopathology reports of Acute and Sub-acute toxicity study showed mild to moderate changes which were non-specific reversible. The histopathology report of some samples of sub-acute toxicity showed mild fatty changes and chronic venous congestion of liver, focal necrosis of brain, which were non-specific and reversible. No significant changes was noted in blood urea of all the samples. The Liver Function Tests of some samples showed mild increase in SGOT, SGPT, Alkaline phosphatase values which may be due to mild fatty changes in liver cells which were non-specific. The Kidney Function Tests of some samples showed mild increase in serum creatinine level which may be due to kidney disorder.

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ISSN: 2456-3110 **ORIGINAL ARTICLE** July-Aug 2019

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How to cite this article: Dr. Amrita Paul, Dr. Umapati C. Baragi, Dr. Kashinath Hadimur, Dr. R. A. Deshmukh. An experiemental study to evaluate the principle Trini Dravyani Nati Upayunjita w.s.r. to Kshara (Alkali). J Ayurveda Integr Med Sci 2019;4:172-181.
http://dx.doi.org/10.21760/jaims.4.4.24

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

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