ELEMENTAL ANALYSIS AND SAFETY OF *YASADA BHASMA* (ZINC ASH)

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INTRODUCTION

- Myanmar Traditional Medicine is famous with four *nayas*
- *Vijjadhara naya* - three major components
- 1. Psycho-spiritual therapy
- 2. Will-power and meditative power
- 3. Metallic medicine

- Metallic medicine - liquid, powder and philosopher' stone (Department of Traditional Medicine, 2008).
- Many types of *bhasma* - zinc ash, tin ash and iron ash, etc
- Zinc ash - urinary disorders, eye disorders, cough, asthma, fever, tremor, epilepsy, migraine, menorrhagia, dysmenorrhea, leucorrhoea, and as *rasayana* drug (Department of Medicine, 2004).

- orally with different vehicles such as honey, milk or ghee (Umrani *et al.*, 2013).
- about 125 mg to 250 mg twice daily (Dash, 1996)
- Very effective with small amount and it is commonly used in clinical practice.
- There is no scientific study about safety of zinc ash.
- Therefore, this study was aimed to carry out elemental analysis and safety of zinc ash.

OBJECTIVES

- To determine the elemental compositions of *Yasada Bhasma* (Zinc Ash)
- To determine the LD_{50} and toxic effect of *Yasada Bhasma* (Zinc Ash) by acute toxicity test
- To determine the toxic effect of *Yasada Bhasma* (Zinc Ash) by sub-acute toxicity test

METHODOLOGY

Study Design

- Laboratory based experimental animal study

Study Period

- 1st July 2017 to 30th June 2018

Study Site

- Department of Medical Research (Pyin-Oo-Lwin Branch)
- Department of Geology, University Research Center, University of Mandalay

Selection Criteria

- Selected in accordance with inclusion criteria.

Acute toxicity test

(a) Inclusion criteria

1. Age: ≥ 6 weeks
2. Weight: 180 ± 20 g
3. Adult healthy albino rats of only male sex
4. Strain : Wister Strain

(b) Exclusion criteria

1. Age: < 6 weeks
2. Weight : >200 g and <160g of rats
3. Female rats and unhealthy rats

Sub-acute toxicity test

(a) Inclusion criteria

1. Age: ≥ 6 weeks
2. Weight: 180 ± 20 g
3. Adult healthy albino rats of both sex
4. Non pregnant (female albino rats)
5. Strain : Wister Strain

(b) Exclusion criteria

1. Age: < 6 weeks
2. Weight : >200 g and <160 g of rats
3. Lactating and Pregnant rats
4. Unhealthy rats

Materials and Methods

Preparation of Zinc Ash

- The zinc ash was prepared by the method of နာဂသေနာဘိဝံသ (၁၉၇၂) and သန်းညွန့် (၂၀၁၃).

Materials for preparation of Zinc Ash

1. Purified zinc
2. Powder of *Achyranthes aspera* L.
3. Iron pan
4. Iron ladle
5. Crucible
6. Special clay
7. Special type of oven

Purification of zinc

- Zinc was made into small pieces and melted by heating.
- At the same time, the milk was also heated.
- After melting, zinc was poured into the milk.
- After cooling down, zinc was taken out and this process was repeated twenty one times to get purified zinc.

Zinc

Purification of zinc

Purified zinc

Preparation of Zinc Ash

- The purified zinc was taken and placed in iron pan. It was melted by heating.
- Then the powder of *Achyranthes aspera* L. was kept on adding in a little quantity at frequent intervals and stirred well with an iron ladle till it turned into powder.
- Afterwards, see that the entire powder was at the center of the iron pan and heated it till became red hot.

- After cooling down, this powder was placed into the crucible and covered with a '*sarava*' and smeared with special clay.
- And then, it was heated by special type of oven until 24 days.
- After it was cooled down, collected zinc ash.
- The obtained zinc ash was stored in a glass bottle and protected from air for experiment.

Melting of purified zinc

***Achyranthes aspera* L.**

Powder of zinc

Preparation of zinc ash

Zinc ash

Elemental analysis of Zinc Ash

- The elemental compositions of the zinc ash was detected by WDXRF and determined by the percentage of sample such as zinc.

Method for Acute toxicity study

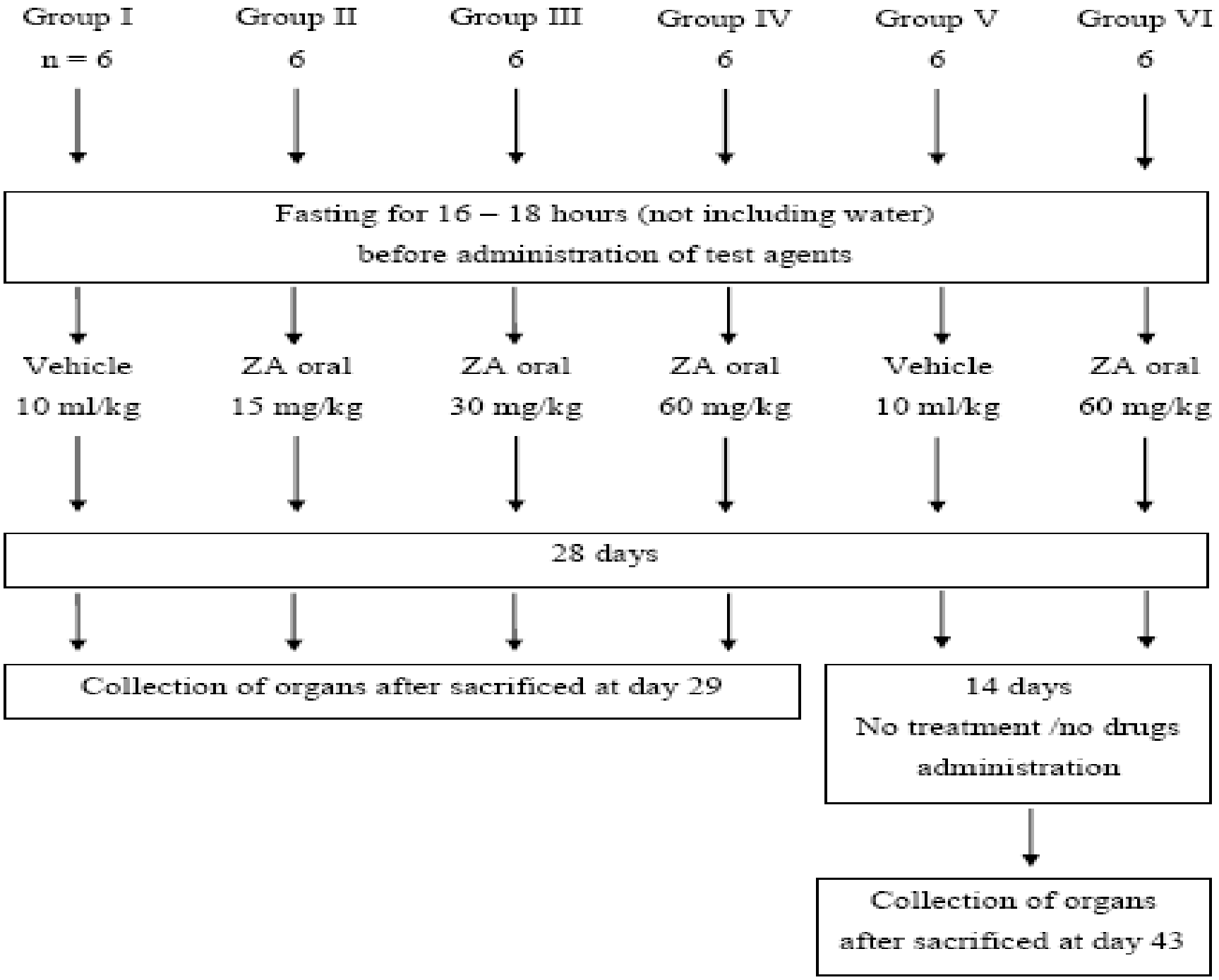
- OECD 425 guideline (2008)
- the main test was performed
- the six male albino rats
- fasted food but not water for 16-18 hours prior to dosing.
- The fasted body weight was recorded and the dose was calculated according to the body weight.

- Dose was initiated at 175 mg/kg.
- After administration, food was withheld for 3-4 hours and toxic changes of rats were observed.
- Each rat was observed carefully for up to 48 hours before making a decision on whether and how much to dose the next animal.

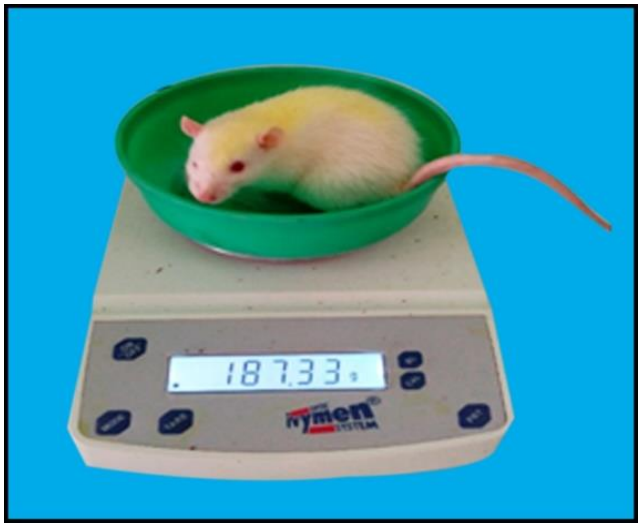
- The dose for the next rat was increased by a factor of 3.2 times the original dose.
- at 175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg were tested in this study
- The clinical observations - at 1/2, 1, 2, 24 hours for 14 days and gross pathology was performed at the end of the study by Loomis and Hayes (1996)

Method for Sub-acute toxicity study

- OECD 407 guideline (2008)
- The different doses were calculated from the human doses (125 mg) by the method of Reagan-Shaw (2007).
- Honey was used as vehicle


Group I
n = 6
Group II
6
Group III
6
Group IV
6
Group V
6
Group VI
6
Fasting for 16 – 18 hours (not including water)
before administration of test agents
Vehicle
10 ml/kg
ZA oral
15 mg/kg
ZA oral
30 mg/kg
ZA oral
60 mg/kg
Vehicle
10 ml/kg
ZA oral
60 mg/kg
28 days
Collection of organs after sacrificed at day 29
14 days
No treatment /no drugs
administration
Collection of organs
after sacrificed at day 43

Six groups of rats



Weighing of rat

Administration of zinc ash

Dissecting of rat

Data Collection and Data Analysis

- analyzed by using SPSS (version 21.0).
- Two-way ANOVA (Repeated Measure Analysis)
- P <0.05 was considered significant.

RESULTS

Elemental Compositions of Zinc Ash

- It was found that zinc oxide (86.2 %) was the most component in the elemental analysis of zinc ash.

No	Elements		Elemental oxide	
	Name	Mass (%)	Name	Mass (%)
1	Aluminium	0.170	Aluminium III oxide	0.321
2	Silicon	1.18	Silicon dioxide	2.52
3	Phosphorus	0.147	Phosphorus pentoxide	0.337
4	Potassium	0.0884	Potassium oxide	0.106
5	Calcium	0.304	Calcium oxide	0.425
6	Chromium	0.0234	Chromium III oxide	0.0342
7	Iron	1.19	Iron II oxide	1.70
8	Copper	0.125	Copper II oxide	0.157
9	Zinc	69.3	Zinc oxide	86.2
10	Arsenic	0.176	Arsenic trioxide	0.232
11	Indium	0.229	Indium III oxide	0.276
12	Tin	3.79	Tin dioxide	4.81
13	Lead	2.64	Lead II oxide	2.85

Result of Acute Toxicity

- Single oral administration of the zinc ash of 175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg did not produce clinical signs of toxicity in all albino rats.
- It was observed that the average body weight gain in all rats after administration of the tested drug.

- There was no lethality of rats up to 14 days observation period.
- There were no changes in clinical observations of parameters, gross features of the organs of the albino rats.
- Therefore, it could be determined that median lethal dose (LD_{50}) of zinc ash was supposed to be greater than 5000 mg/kg.

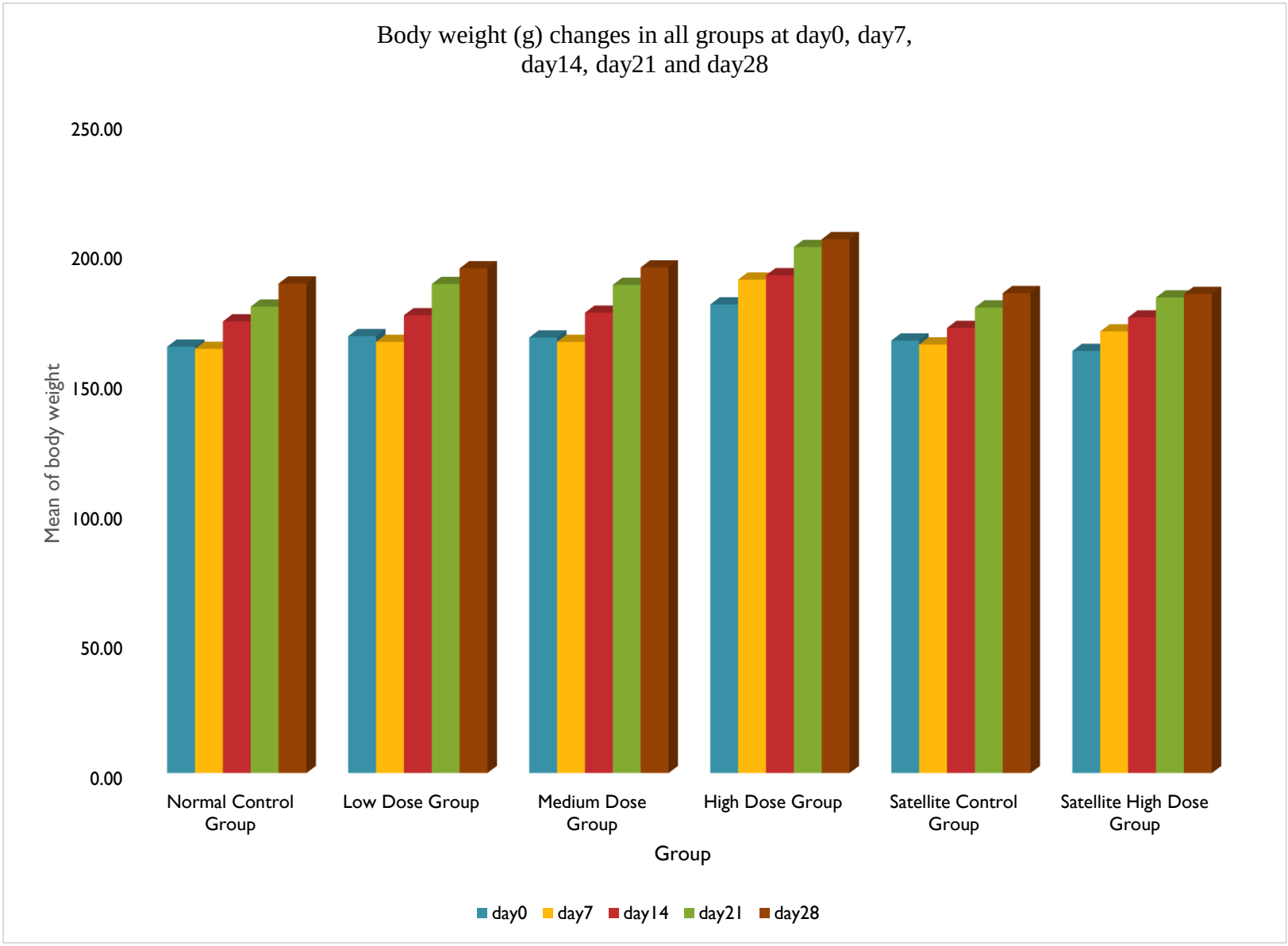
Result of Sub-acute Toxicity Study

- The clinical toxic signs and mortality did not observe in all albino rats for 28 days.
- There was significant body weight gain in all rats of control and tested groups after 28 days.
- There were also no delayed effects of zinc ash in satellite groups.

- Gross features of lung, heart, liver, stomach, spleen, and kidney in all groups did not show any morphological difference when compared with the control group.
- The shape, size and texture of these collected organs of tested rats were found to be normal.

- Histopathological features in all groups - lung, heart, liver, kidney, stomach and spleen were revealed no abnormalities.
- Mild congestion of capillaries and blood vessels were observed in kidney and liver of medium and high groups.
- Other organs revealed no failure on comparison with the control group

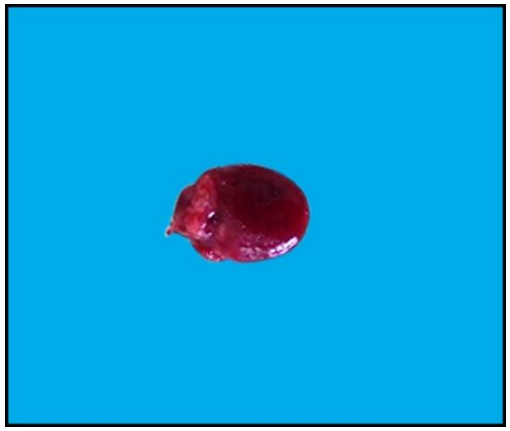
zinc ash.



Mean body weight changes of rats in all groups

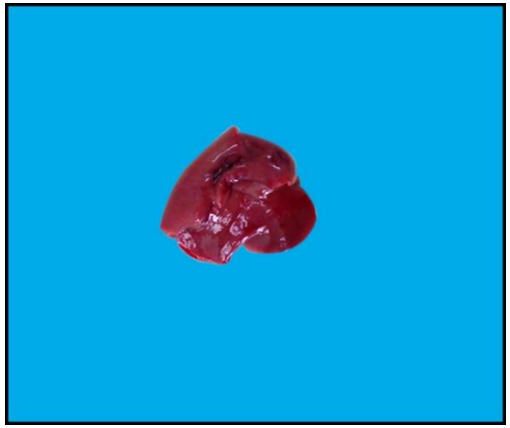
Gross features of the organs of rats

Lung

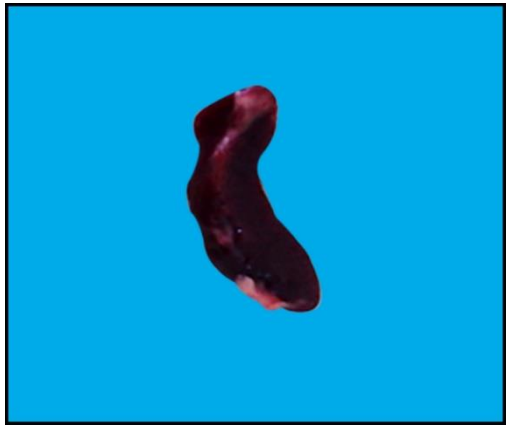


Heart

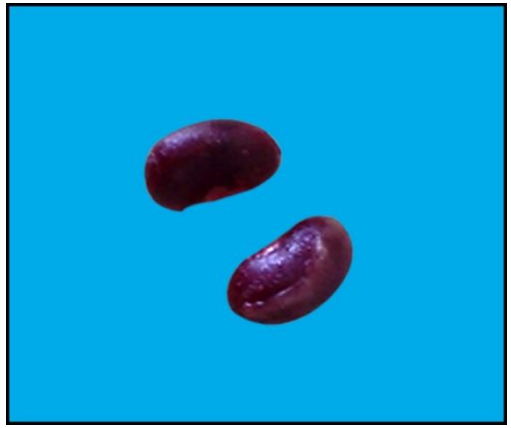
Stomach



Liver

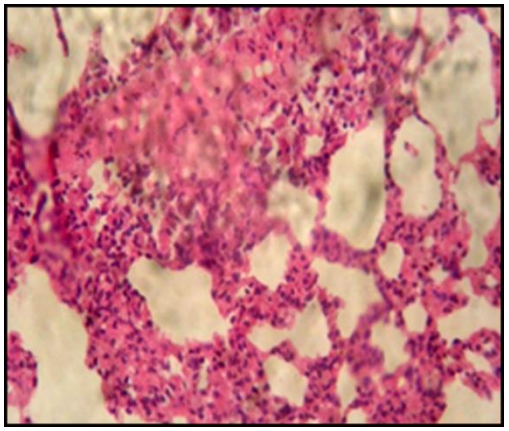


Spleen

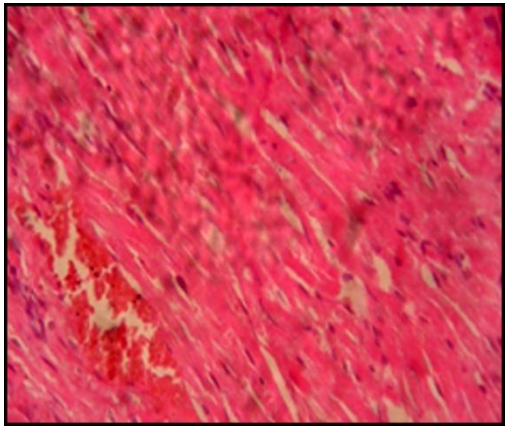


Kidneys

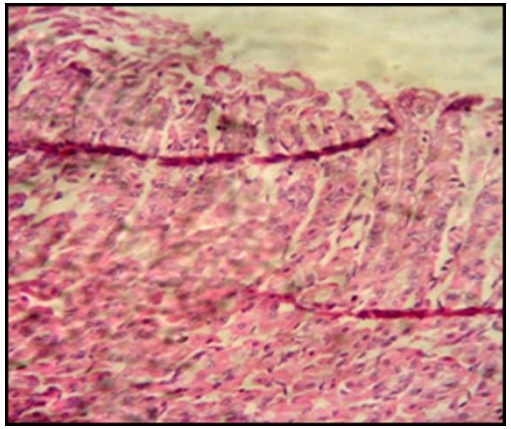
Histopathological features of the Organs



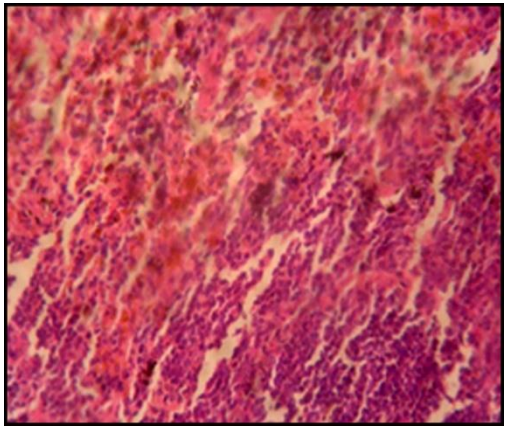
Histology of Lung



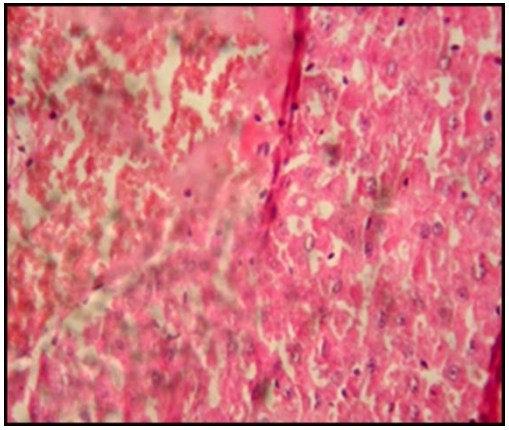
Histology of Heart



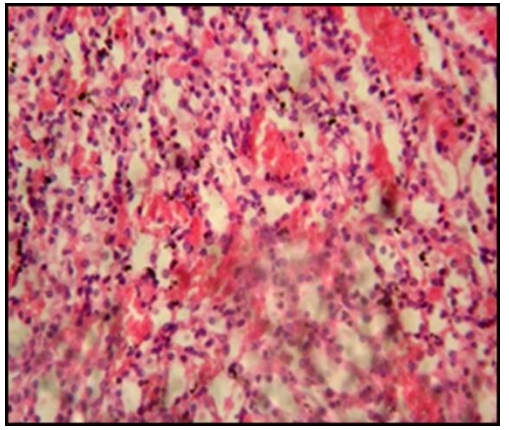
Histology of Stomach



Histology of Spleen



Few dilated vessels with mild vascular congestion in histology of liver



Thickened arterial walls and aggregates of inflammatory cells in histology of kidney

DISCUSSION

- The elemental compositions may be dependent upon the purification method of zinc, the preparation method and heating duration.
- The body weight of control and tested groups increased.
- Therefore, the body weight gain of rats may not be adverse effect of zinc ash.

- Histopathological features - the mild histopathological changes were observed in kidney and liver of medium and high dose groups
- These findings were not sufficient to a diagnosis of toxicity.
- Therefore, zinc ash was found to be free of any toxic effects under the condition of this study and may be safe as oral administration in short term.

CONCLUSION

- Elemental analysis - zinc oxide (86.2%) was found as main component
- Zinc deficiency - mental disorders (madness), poor memory and neurological disorders in *Vijjadhara Naya*
- Therefore, zinc ash can be used in these diseases.

- Acute toxicity - LD_{50} of zinc ash is greater than 5000 mg/kg
- Sub-acute toxicity - no significant changes in histopathological findings even the highest dose (60 mg/kg) for 28 days

- Showed that no toxic effects in oral medication of zinc ash for acute and sub-acute toxicity on albino rats
- According to the findings of this study, can be used under the dose of 500 mg/kg body weight in human.

SUGGESTIONS

- Based on the experimental results of this study, further study should be carried out
- by the other different method of purification of zinc, preparation method and heating duration
- for chronic toxicity study to ensure long term safety

- experimental animal study for therapeutic uses
- for safety and efficacy of other metallic medicine commonly used in Myanmar Traditional Medicine

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- သန်းညွန့်(ဦး)(၂၀၁၃)၊ သမားလက်သုံးဓာတ်ဆေးပညာအဖွင့်ကုထုံးကျမ်း၊ ပထမအကြိမ်၊ ပထမတွဲ-ဒုတိယတွဲ၊ ဝင်းရတနာအော့ဖ်ဆက်(မြဝ၁၇၉၂)၊ မန္တလေး။
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Thank You So Much