PHYTOCHEMICAL ANALYSIS AND TOXICITY STUDY OF *THWAY-ARR-TOE-HSEI:* (ASM-16) ON ALBINO RATS

Nang Aye Moe Khaing[1], Zin Mar Lwin[1], Khin Moe Aung[2], Win Yu Aung[1], Myintzu Tin Oung[2], Kyi Kyi Oo[1] &
Thin Lae Win[1]

1. University of Traditional Medicine, Mandalay
2. Department of Medical Research (Pyin Oo Lwin Branch)

INTRODUCTION
OBJECTIVES
METHODOLOGY
DATA ANALYSIS
RESULTS
DISSCUTION
CONCLUSION
SUGGESTIONS
REFERENCES
ACKNOWLEDGEMENT

INTRODUCTION

- Anemia - a hemoglobin concentration lower than 12 g/dl in women and 13 g/dl in men (WHO, 2011)
- Aneamia - public health problems
- Prevalence of anaemia - greater than 40% in Myanmar children aged 6 - 59 months and 20 - 39.9% in females aged 15 - 49 years (WHO, 2015)

- There are many formulations for the treatment of anaemia. Many types of blood tonic are *Dei-Wa-O:Tha-Da-Thway-Hsei:* (TMF-14), *Thee-Chae-Hsei:* (TMF-15), *Apu-Nyein-Thway-Hsei:* (TMF-16), *Thway-Hsei:-Ni* (TMF-17), *Mahar-kalja-Ni-Hsei:* (TMF-20), *A-Bei-Njin-Hna-Loun-Thway-Arr-Toe-Hsei:* (TMF-80), *Thu-kha-Sheeta-thee-Chae-Hsei:*, *Thway-Arr-Toe-Hsei:* (ASM-16) and *Thway-Hsei-Ngan*. They treat for anaemia.

- ASM-16 has been used in Traditional Medicine Teaching Hospital and Traditional Medicine clinic since 2003.
- Evidence based safety profiles of Traditional Medicine Formulations are needed
- No scientific report of toxicity studies of *Thway-Hsei:*
- Investigated for acute and sub-acute toxicities of *Thway-Arr-Toe-Hsei*: (ASM-16) in animal model by OECD guidelines.

OBJECTIVES

1. To determine the phytochemical analysis of *Thway-Arr-Toe-Hsei:* (ASM-16)
2. To find out the physico-chemical analysis of *Thway-Arr-Toe-Hsei:* (ASM-16)
3. To identify the elemental analysis of *Thway-Arr-Toe-Hsei:* (ASM-16)
4. To evaluate the acute and sub-acute toxicity of *Thway-Arr-Toe-Hsei:*(ASM-16) on albino rats

METHODOLOGY

Study Design - Laboratory based experimental animal study

Study Period - 1st September 2016 to 31st August 2017.

Study Area - Research Division, University of Traditional medicine

- Department of Physics, Mandalay University
- Department of Medical Research (Pyin Oo Lwin Branch).

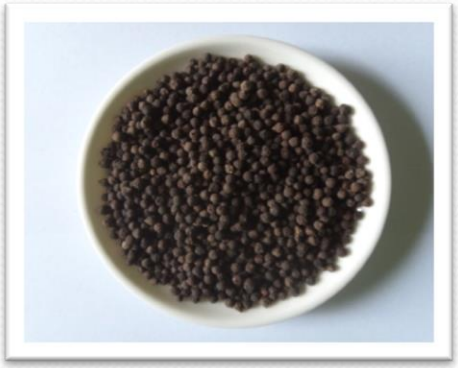
1. Preparation of ASM-16

No.	Myanmar Name	Scientific Name	Part used	Weight			Percentage
				Myanmar Unit		Metric Unit	
				Kyat	Pe	Gram	
1.	Na-tha-ni	*Pterocarpus santalinus* Linn.	Wood	2	-	32	3.36 %
2.	Na-tha-hpju	*Santalum album* Linn.	Wood	2	-	32	3.36 %
3.	Pan:-nu	*Saussurea affinis* Spreng.	Rhizome	2	-	32	3.36 %
4.	Ei'-mwei-thi:	*Embelia robusta* Roxb.	Seed	2	-	32	3.36 %
5.	Kja- thee- zan	*Nelumbo nucifera* Gaertn.	Seed	2	-	32	3.36 %
6.	Mjin:-khwa	*Centella asiatica* Linn.	Leave	2	-	32	3.36%
7.	Kja-zu-thee	*Terminalia citrina* Roxb.	Fruit	2	-	32	3.36%
8.	Kjau'-thwei:	Ferric ammonium citrate	Mineral	2	4	36	4.04%
9.	Taun-kja-kje'-thwei	*Stephania venosa* Blume.	Rhizome	4	-	64	6.73%
10.	Sei:-makhan:	*Jatropha multifida* Linn.	Stem	4	-	64	6.73%
11.	Zi:-hpju-thi:	*Emblica officinalis* Linn.	Fruit	5	-	80	8.41%
12.	Thi'-hsein	*Terminalia bellerica* Roxb.	Fruit	5	-	80	8.41%
13.	Gan.-go-wu'-hsan	*Mesua ferrea* Linn.	Stamen	5	-	80	8.41%
14.	Nwe-gjou	*Glycyrrhiza glabra* Linn.	Stem	5	-	80	8.41%
15.	Dha-gja:	*Saccharum officinarum*	-	5	-	80	8.41%
16.	Kja-wu'-hsan	*Nelumbo nucifera* Gaertn.	Stamen	10	-	160	16.83%
Total weight				**59**	**4**	**948**	**99.9%**

(Na-tha-ni)

Na-tha-hpju

Pan:-nu



Ei’-mwei-thi:t

Kja- thee- zant

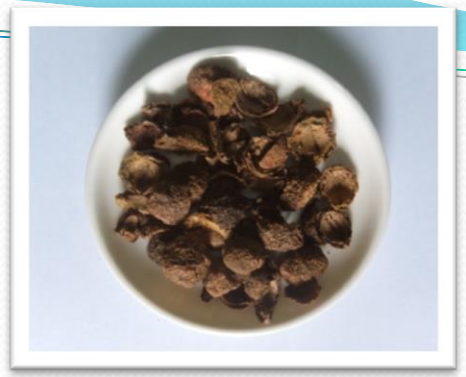
Mjin:-khwa

Kja-zu-theeti

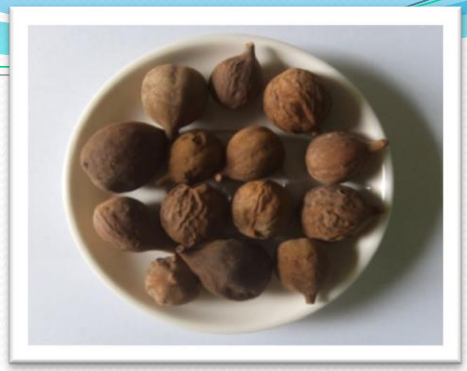
Kjau’-thwei:

Taun-kja-kje’-thwei

Sei:-makhan:



Zi:-hpju-thi:



Thi’-hsein

Gan.-go-wu’-hsan

Nwe-gjou

Dha-gja:

Kja-wu’-hsan

Powder of ASM-16

2. Methods for phytochemical analysis

- Carried by method of Harbone (1998) and Raaman (2006)

3. Methods for physico-chemical analysis

- Analyzed by WHO (2011)

4. Method for elemental analysis

- Analyzed by using energy dispersive x-ray fluorescence system(EDXRF)

5. Method for acute toxicity study

- OECD 425 guideline (2008)
- the main test - the test agent is likely to be non-toxic
- Initiated at 175mg/kg
- Next animal was increased by dose factor 3.2 times 550 mg/kg
- at 2000 mg/kg body weight
- A single dose was calculated according to the body weight of rats

Method for Acute Toxicity study

Five ICR strain mice in each groups were kept in the cages

G -1	G-2	G-3	G-4	G-5

prior to dosing for 16-18 hours and were weighted

ASM-16 175mg/kg	ASM-16, 550 mg/kg	ASM-16, 2000 mg/kg	ASM-16, 2000 mg/kg	ASM-16, 2000 mg/kg

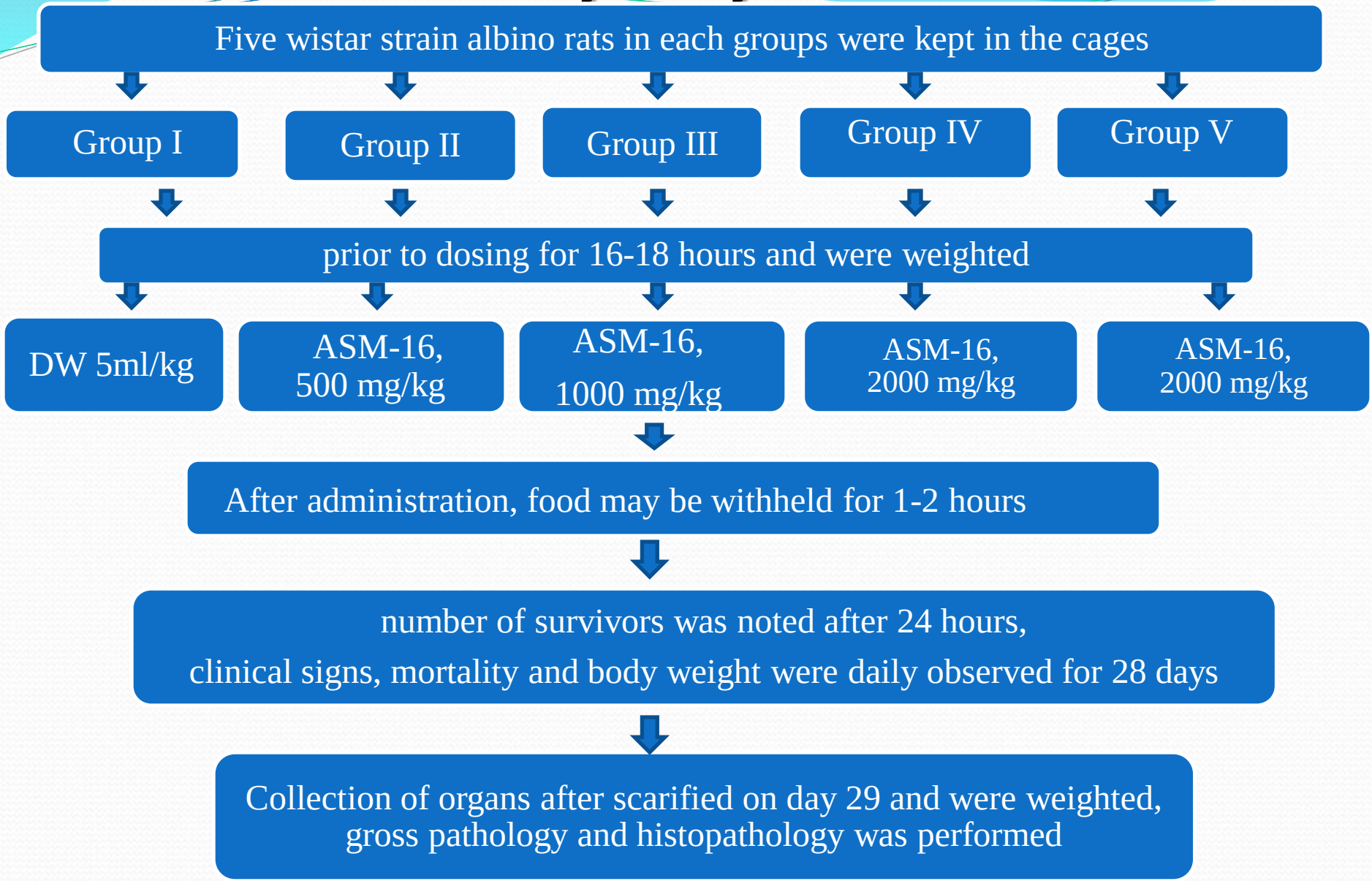
After administration, food may be withheld for 1-2 hours

number of survivors was noted after 24 hours,
clinical signs, mortality and body weight were daily observed for 14 days

- The following clinical observations:

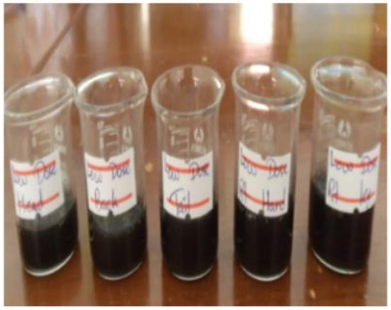
  skin, fur, eyes, mucous membrane, salivation, respiratory rate, motor activity, paralysis of limbs, behavioral pattern, tremor, convulsion, diarrhea and mortality were assessed at 1/2, 1, 2, 24 hours for 14 days and gross pathology was performed at the end of the study

6. Sub-Acute Toxicity study

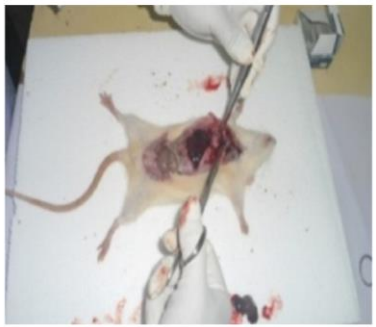


Weighing machine for rats

Five Groups of rats

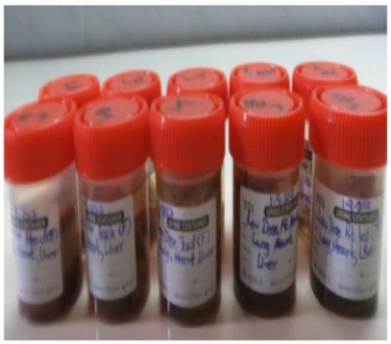


Preparation of Drugs



Dissecting of rats

Oral administration of ASM-16



Fixation with formalin

Cutting of organ

Tissue casettes

Tissue processing

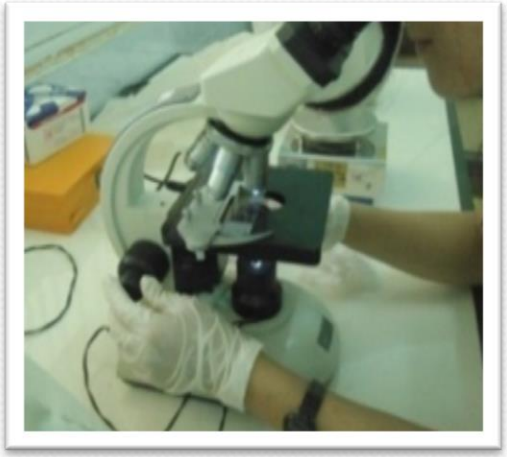
Tissue Processor (Shandon Citadal 2000)

Wax dispenser

Microtome (Model- SLEE cut 4055)

Staining with Eosin and Haematoxylene

Oven



Microscope

Data analysis

- analyzed for statistically using by SPSS (version 21.0)
- One way ANOVA test - to observe the significance of difference test group and compare with respective control group $P < 0.05$ was considered significant

RESULTS

1. Phytochemical analysis of ASM-16

- observed that the alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, steroids, reducing sugar, carbohydrates, amino acid, tannin and saponins were present
- but cyanogenetic glycosides were absent

2. Physico-chemical properties of ASM-16

No	Physicochemical Parameters	Quantity determined Percentage
1	Moisture content	4.7 %
	(Loss on drying of 105^{o}C)	
2	pH values	
	- 1% of solution	5.7 %
	- 10% of solution	5.5 %
3	Total ash	5.4 %
	- Acid insoluble ash	1.2 %
	- Water soluble ash	96.7 %
4	Solubilities	
	- Water soluble matter	24.4 %
	- Ethanol soluble matter	16.2%

3. Elemental compositions of ASM-16

No.	Macro Elements	Percentage
1.	K	0.9660
2.	Cl	0.8709
3.	Ca	0.5579
4.	P	0.1872
5.	Si	0.0834
6.	Al	0.0505

No.	Micro Elements	Percentage
1.	Fe	0.6300
2.	Ca	0.5579
3.	Si	0.0834
4.	Zn	0.00253
5.	Mn	0.0093

4. Acute Toxicity Study of ASM-16

- 175 mg/kg, 550 mg/kg, 2000 mg/kg, 2000 mg/kg, 2000 mg/kg
- Clinical observations - no abnormality detected
- Grossly features of rats (Lungs, Heart, Stomach, Liver, Spleen and Kidneys) are normal
- Neither haemorrhage nor necrosis, congestion were noted on cut sections median
- lethal dose (LD_{50}) of ASM-16 was supposed to be greater than 2000 mg/kg.

5. Sub-acute toxicity study of ASM-16

- The following clinical observations:

  skin, fur, eyes, mucous membrane, salivation, respiratory rate, motor activity, paralysis of limbs, behavioral pattern, tremor, convulsion, diarrhea and mortality were assessed at 1/2, 1, 2, 24 hours for 28 days

- Gross features of the organs are normal
- There was no significant change in body weight before and after administration of the test drug.

• Organ weight (Mean ± SE) of control group and treated groups as 500 mg/kg, 1000 mg/kg, 2000 mg/kg and 2000 mg/kg (satellite) were no statically significant in organ weight

Histopathological features

(1) Lungs

Alveoli - There is no feature of necrosis. In high dose group was mild congestion of alveoli capillaries with mononuclear cell infiltration.

(2) Heart

Sections of cardiac muscle of all groups show striated fiber with a single (central) nucleus. There is no feature of necrosis.

Histopathological features

(3) Stomach

There is no feature of necrosis.

(4) Liver

There is no feature of necrosis. There were congestion of central vein and infiltration of mononuclear cells in high dose group.

Histopathological features

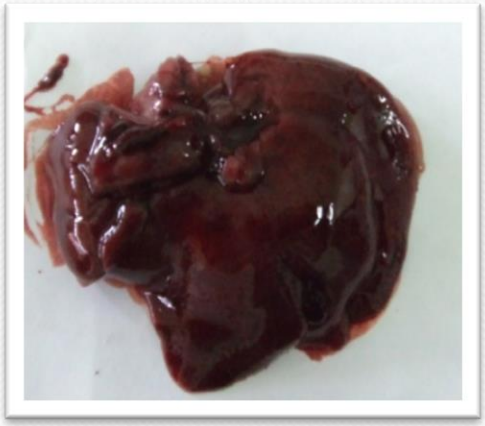
(5) Spleen

There is no feature of necrosis.

(6) Kidneys

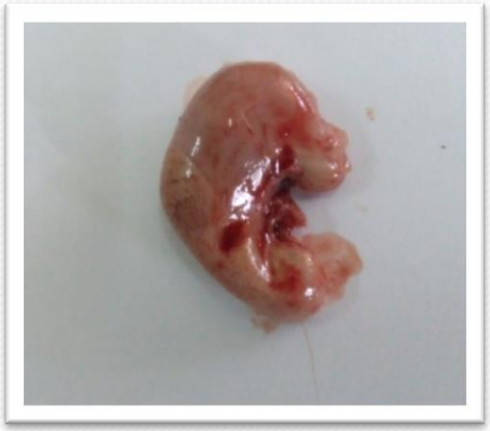
there were mild congestion and infiltrations of mononuclear cells were noted in some glomeruli of high dose group.

Internal Organs of the Rats

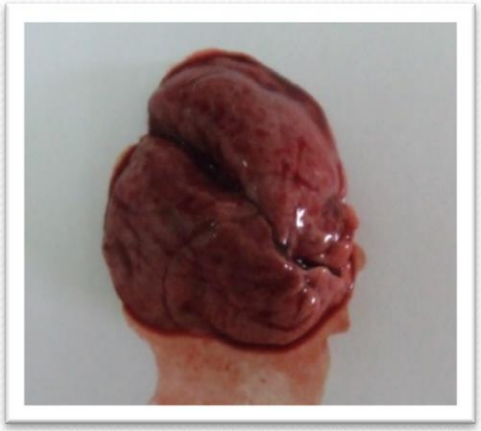


Liver

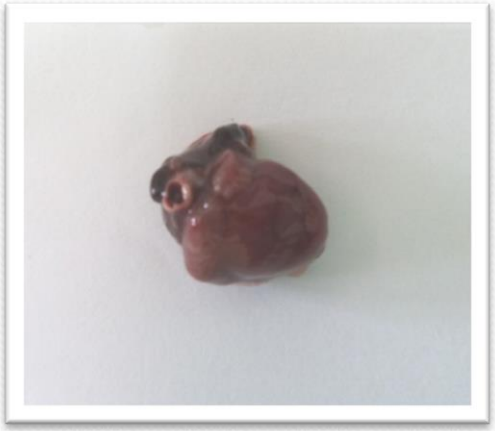
Spleen



Stomach



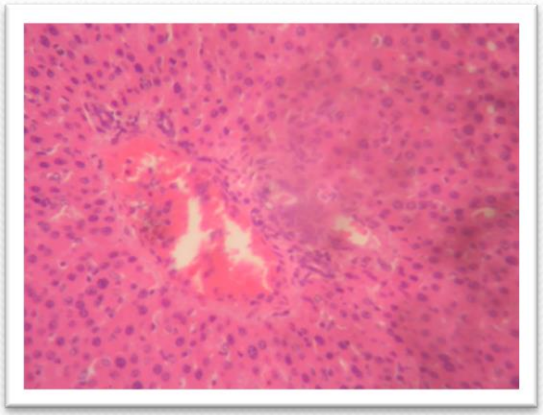
Lungs



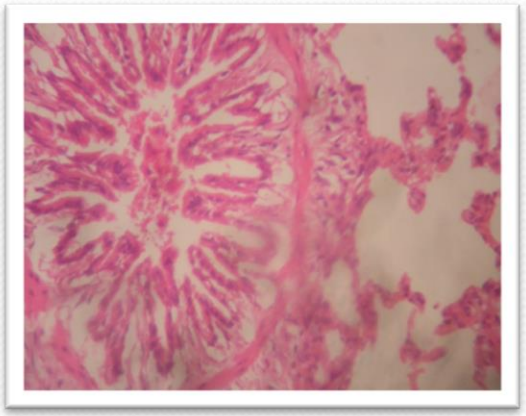
Heart

Kidney

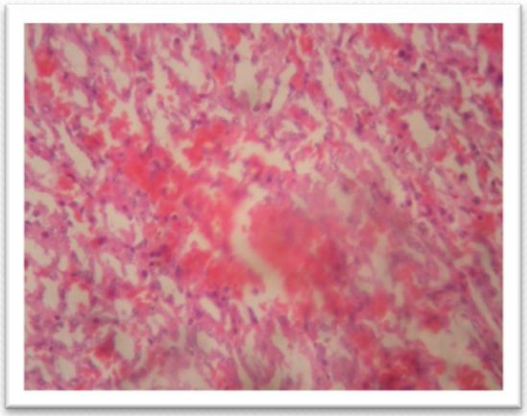
Histopathological features



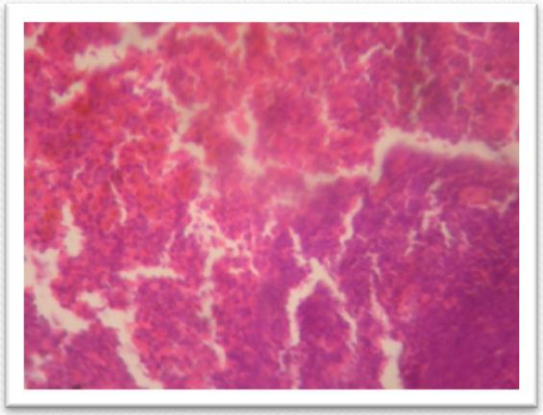
Liver



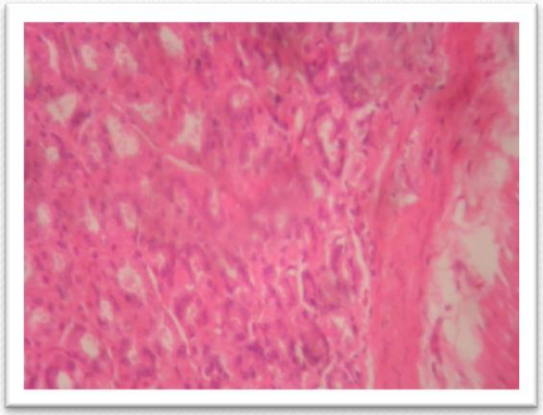
Lungs



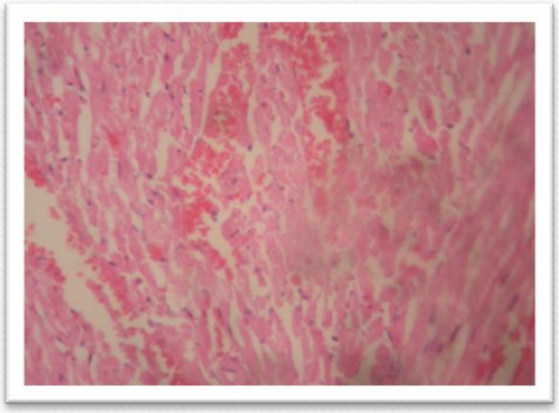
Kidney



Spleen



Stomach



Heart

Discussion

- Quality control methods for medicinal plant materials
- were observed that the alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, steroids, reducing sugar, carbohydrates, amino acid, tannin and saponins were present but cyanogenetic glycosides were absent

- Moisture content- 4.66 %
- (no more than 14%)
- deteriorated due to fungus and bacterial growth / prevent content bacterial, fungal or yeast growth through storage
- Total ash -5.41 %
- (European pharmacopoeia- 14%)
- also reflects the care taken in drug preservation, and the purity of crude and the prepared drug

- higher concentration of Potassium (0.9660%), Chlorine (0.8709%), Iron (0.6300%), Calcium (0.5579%), Phosphorous (0.1872%), Silicon (0.0834) and Aluminum (0.0505%) were observed in this study

- oral medication without any toxic effects under the dose of 2000 mg/kg body weight on acute and sub-acute toxicity study on albino rats.

CONCLUSION

- Present of saponins, phenolics and glycosides may be responsible for anti-anaemic properties
- Total ash and acid insoluble ash values determinations are also particularly important in the evaluation of purity of drugs that is the presence or absence of foreign organic matter.

- Micro and macro-elements has revealed that its rich and good source of K, Cl, Fe, Ca and P. These elements do provide important constituents for different body metabolic enzymes for human and essential elements for human health.
- Iron is necessary for the formation of haemoglobin
- Also plays an important role in oxygen and electron transfer in human body
- K - important as diuretic and it takes part in ionic balance of the human body and maintains tissue excitability

- Showed that there were no toxic effects in oral medication of ASM-16 for acute and sub-acute toxicity.
- Should be used under the dose with 2000 mg/kg body weight
- Further studies should be carried out for efficacy and chronic toxicity with large samples size.

SUGGESTIONS

- Standardization tests should be done for quality control (QC) and quality assurance (QA) purpose
- Further study should be carried out for chronic toxicity

  Clinical trial research works are necessary to conduct for the evaluation of efficacy

ACKNOWLEDGEMENT

ဤစာတမ်းကိုဖတ်ကြားခွင့်ပြုပါသော အားကစားနှင့်၊ ကျန်းမာရေးဝန်ကြီးဌာန ကျန်းမာရေးဝန်ကြီး၊ တိုင်းရင်းဆေးပညာဦးစီးဌာန ညွှန်ကြားရေးမှူးချုပ်၊ တိုင်းရင်းဆေးတက္ကသိုလ်ပါမောက္ခချုပ်၊ စာတမ်းကြီးကြပ်ပေးပါသော ဆရာ၊ ဆရာမများ ဤစာတမ်းတွင် အဖက်ဖက်မှ ဝင်းဝန်းကူညီဆောင်ရွက်ပေးပါသော ဆရာ၊ ဆရာမများအားလုံးကို အထူးကျေးဇူးတင်ပါသည်။

REFERENCES

- Organization for Economic Co-operation and Development (OECD) (1995).Repeated Dose 28-Day Oral Toxicity Study in Rodents, In: OECD Guideline for Testing of Chemicals 407. 1-7.
- Organization for Economic Co-operation and Development (OECD). (2008). Acute oral toxicity- Up and down procedure. In: OECD Guideline for Testing of Chemicals 425. 1-13.

- Raaman N. (2006). Phytochemical techniques, New India Publishing Agency, Pitam Pura, New Delhi.
- Reagan-Shaw S., Nihal M. and Ahmad N. (2007). Dose translation from animal to human studies revisted, vol 22: 660.
- Smith A. (2012). Guide to evaluation and treatment of anaemia in general practice. Drug review anaemia. 25-38.

- World Health Organization. 2015. The global prevalence of anaemia in 2011, WHO Library Cataloguing In Publication Data. Geneva, Switzerland.
- World Health Organization. 2011. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva.
- World Health Organization. 2011. Quality control methods for herbal materials. WHO Press. Geneva, Switzerland.

Thank You So Much