ACUTE AND SUB-ACUTE TOXICITY OF *THWAY- TOE- KYA- HSEI* (AHD-9) OF TRADITIONAL MEDICINE TEACHING HOSPITAL, MANDALAY, IN RATS

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OBJECTIVES

1. To determine the acute and sub-acute toxicity of *Thway-Toe-Kya-Hsei* (AHD -9) of Traditional Medicine Teaching Hospital, Mandalay in rats
2. To evaluate the safety profile by acute and sub-acute toxicity data of AHD-9 in rats

MATERIALS AND METHODS

Bommayarzar

Kyat Thun Phyu

NantTharNi

SinToneMaNwe

Ayekarit

Thanakhar

NantTharPhyu

SaungMayKhar

Nanwin Kharr

Materials For Acute Toxicity and Sub-acute Toxicity

- Fine powder of *AHD-9*
- Distilled water
- Wistar strain rats weighting150±50g
- Cages
- Work sheet
- Beaker
- Mask
- Measuring cylinder
- Glove
- Syringe

- Weighing machine for rats
- Weighing machine for drug
- Formalin
- Tissue cassettes
- Tissue Processor (Shandon Citadal 2000)
- Wax dispenser
- Microtome (Model- SLEE cut 4055)
- Eosin and Haematoxylene
- Oven
- Microscope
- Cannula

PREPARATION OF AHD -9

- The raw materials were collected from Mandalay Herbal Market
- They were carefully washed with tap water and air-dried under shade
- They were chopped into small pieces and made powder by using grinding machine
- They were stored into the air tighten bottles at room temperature

THWAY-TOE-KYA-HSEI (AHD-9)

ACUTE TOXICITY AND SUB-ACUTE TOXICITY

- Acute oral toxicity and sub-acute toxicity of AHD-9 were done at the Department of Medical Research (Pyin Oo Lwin Branch) in 2015 by experimental based study

METHODS FOR ACUTE TOXICITY STUDY

- Acute oral toxicity of *AHD-9* was carried out according to OECD 425 guideline (2008)
- In this study, the limit test was selected because the test agent is likely to be non-toxic
- The albino rats were kept in the cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions with unlimited supply of food and water.

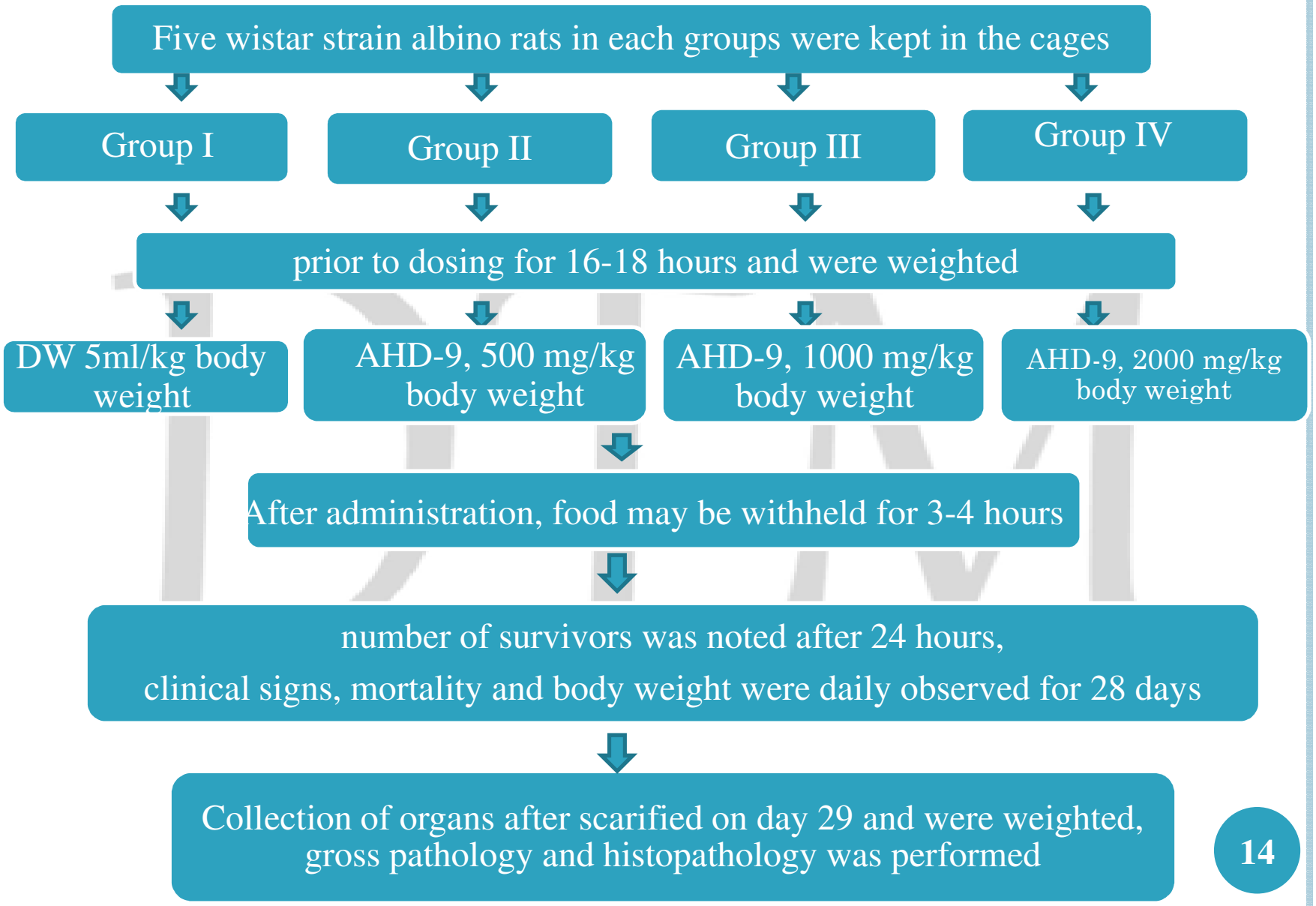
- The rats were fasted food but not water for 16-18 hours prior to dosing
- The fine powder of drug was dissolved in distilled water for required concentration to be administered, at 5000 mg/kg body weight
- A single dose was calculated according to the body weight of rats

- After administration, food may be withheld for 3-4 hours
- 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 by Loomis and Hayes (1996)

SUB-ACUTE ORAL TOXICITY

- A repeated dose oral toxicity study was conducted as per OECD guideline 407, on the four groups of rats and 5 rats in each group (1 male and 4 female)
- Distilled water was used as vehicle

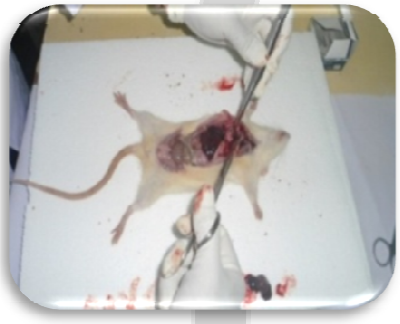
METHODS OF SUB-ACUTE TOXICITY



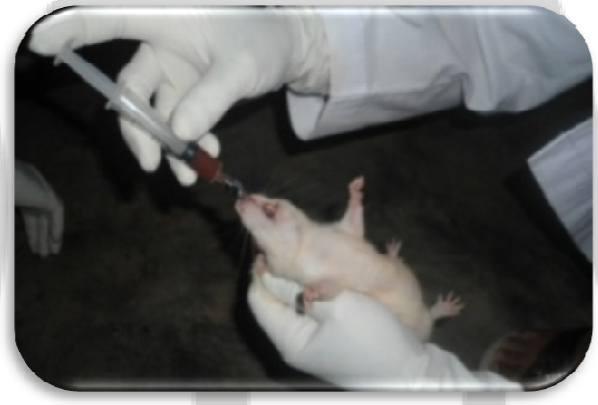
Weighing machine for rats

Four Groups of

Preparation of Drugs



Dissecting of rats



Oral administration of AHD-9

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

- The arithmetic means(m), standard deviation (SD), standard error (SE) and One way ANOVA tests were used by using SPSS (version 21) to observe the significance of difference among groups and compare with test groups and control group $P < 0.01$ was considered significant

RESULTS & DISCUSSION

Acute Toxicity Study of AHD-9

- There was no lethality at 5000 mg/kg body of the AHD-9 in rats for 14 days
- Therefore, median lethal dose (LD_{50}) was determined greater than 5000 mg/kg body weight according to OECD guideline
- There was no abnormality detected
- Grossly features of rats (Lungs, Heart, Stomach, Liver, Spleen and Kidneys) are normal
- Neither haemorrhage nor necrosis was noted on cut sections

Responses of rats during 28 days observation period after administration of repeated oral dose

Parameters	Responses
Skin and fur changes - Skin changes - Piloerection	 NAD NAD
Eyes - Lacrimation - Corneal reflex - Pupillary reaction to light	 NAD NAD NAD
Mucous membrane	NAD
Salivation	NAD
Respiratory Rate	NAD
Motor activity	NAD
Paralysis of limbs	Absent
Behavioral pattern	NAD
Tremor	Absent
Convulsion	Absent
Diarrhoea	Absent

HISTOPATHOLOGICAL FEATURES

- Sections of lungs tissue of all groups show the appearance of fine lace because most of the lungs are composed of thin-walled alveoli
- The alveoli are composed of a single layer of squamous epithelium and no necrosis in lungs
- Sections of cardiac muscle of all groups show striated fiber with a single (central) nucleus and no observed necrosis feature in heart

- Sections of stomach tissue of all groups show mucosa, submucosa, muscularis propria and serosal layers
- Mucous secreting mucosal and submucosal glands lined with single layer of cuboidal epithelium
- There is no feature of necrosis
- Section of liver of all groups consists of lobules
- The center of the lobule is the central vein

- At the periphery of the lobule are portal triads and necrosis feature was not performed
- Section of spleen of all groups consists of two main types of tissue
- They are white pulp which contains lymphoid aggregations, mostly lymphocytes and red pulp which contains vessels
- There is no feature of necrosis
- Section of kidneys of all groups consists of numerous glomeruli and tubule which are lined by eosinophilic (pink) low columnar cells and have not seen feature of necrosis

SUB-ACUTE TOXICITY STUDY OF AHD-9

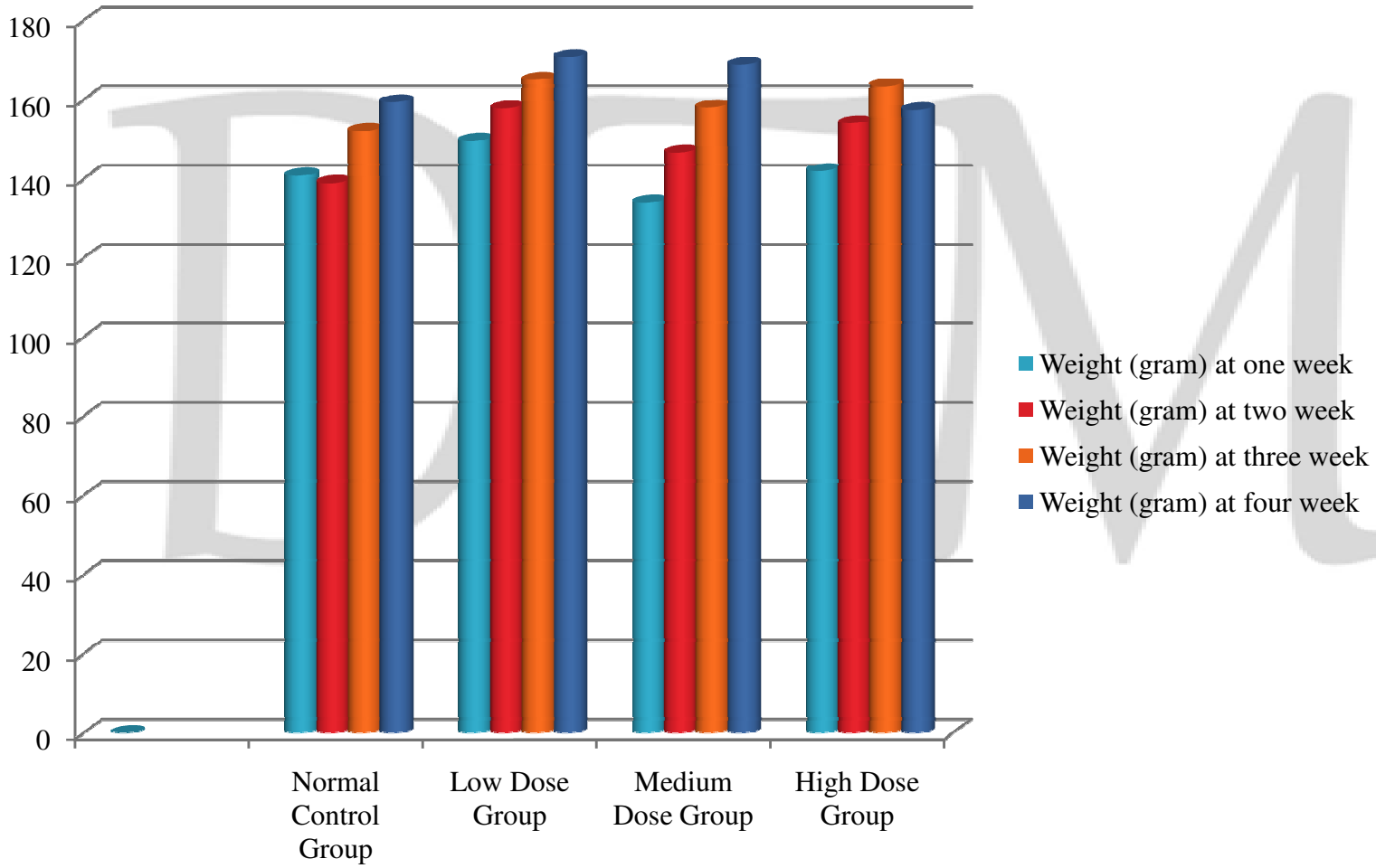
- There was no toxicity sign, mortality, significant difference body weight, grossly and histopathology changes of organs (heart, lungs, liver, spleen, kidneys, stomach)
- The NOAEL of AHD-9 was estimated greater than 2000 mg/kg/ body weight
- Hence, it can be concluded that AHD-9 is safe for oral administration
- There was no abnormality detected

- There was no significant change in body weight before and after administration of the test drug
- Gross features of the organs are normal
- Neither haemorrhage nor necrosis was noted on cut sections

RESPONSES OF RATS DURING 28 DAYS OBSERVATION PERIOD AFTER ADMINISTRATION OF REPEATED ORAL DOSE

Parameters	Responses
Skin and fur changes - Skin changes - Piloerection	 Absent Absent
Eyes - Lacrimation - Corneal reflex - Pupillary reaction to light	 NAD NAD NAD
Mucous membrane	NAD
Salivation	NAD
Respiratory Rate	NAD
Motor activity	NAD
Paralysis of limbs	Absent
Behavioral pattern	NAD
Tremor	Absent
Convulsion	Absent
Diarrhoea	Absent

Comparison Of Weight (Gram) Of Rats In Test Intervals



Mean Score For Weight Of All Groups At One Week, Two Week, Three Week And Four Week

Groups	Mean Score for Weight			
	One week	Two week	Three week	Four week
Control Group	140.8	139	152	159.4
Low dose Group	149.6	157.8	165	170.8
Medium dose group	134	146.6	158	168.8
High dose group	142	154.6	163.4	157.4

Comparison of weight (g) of each group at one week, two week, three week and four week

Comparison of weight (gram)		Paired Differences					T	df	Sig.2 tailed
		Mean	SD	SE	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Weight (g) at one week	-139.1	16.2	3.6	-146.7	-131.5	-38.4	19	.00
Pair 2	Weight (g) at two week	-146.9	16.5	3.7	-154.6	-139.6	-39.9	19	.00
Pair 3	Weight (g) at three week	-157.1	21.7	4.9	-167.3	-146.9	-32.3	19	.00
Pair 4	Weight (g) at four week	-161.6	16.7	3.7	-169.4	-153.8	-43.4	19	.00

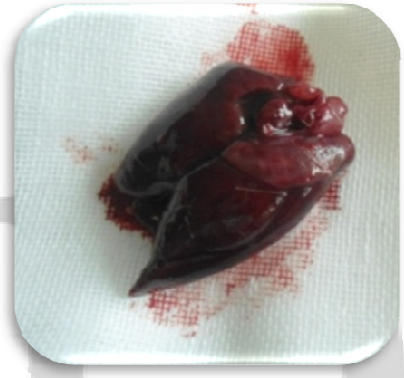
Histopathological features of control and AHD-9 treated rats

Tissue	Treatment	Male	Female
Liver	Control Group 5 ml/kg	Normal	Normal
	TTKH 500mg/kg	Normal	Normal
	TTKH 1000mg/kg	Normal	Normal
	TTKH 2000mg/kg	Normal	Normal
Spleen	Control Group 5 ml/kg	Normal	Normal
	TTKH 500mg/kg	Normal	Normal
	TTKH 1000mg/kg	Normal	Normal
	TTKH 2000mg/kg	Normal	Normal
Heart	Control Group 5 ml/kg	Normal	Normal
	TTKH 500mg/kg	Normal	Normal
	TTKH 1000mg/kg	Normal	Normal
	TTKH 2000mg/kg	Normal	Normal

Histopathological features of control and AHD-9 treated rats

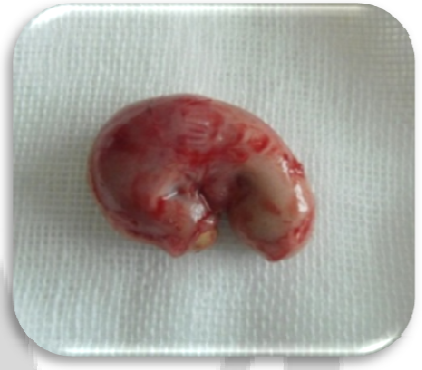
Tissue	Treatment	Male	Female
Lungs			
	TTKH 500mg/kg	Normal	Normal
	TTKH 1000mg/kg	Normal	Normal
	TTKH 2000mg/kg	Normal	Normal
Kidney	Control Group 5 ml/kg	Normal	Normal
	TTKH 500mg/kg	Normal	Normal
	TTKH 1000mg/kg	Normal	Normal
	TTKH 2000mg/kg	Normal	Normal
Stomach	Control Group 5 ml/kg	Normal	Normal
	TTKH 500mg/kg	Normal	Normal
	TTKH 1000mg/kg	Normal	Normal
	TTKH 2000mg/kg	Normal	Normal

INTERNAL ORGANS OF THE RATS

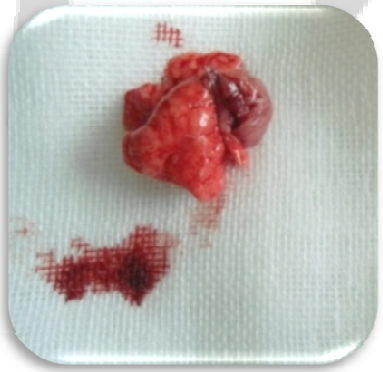


Liver

Spleen

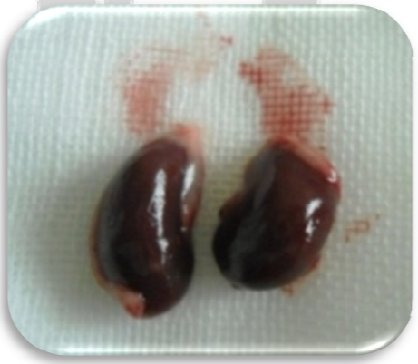


Stomach



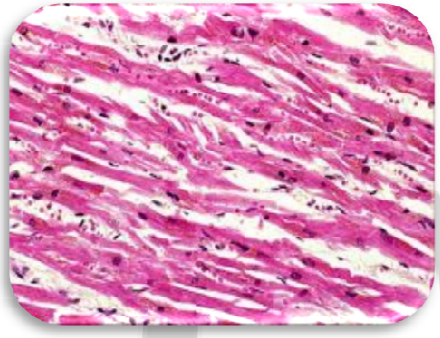
Lungs

Heart

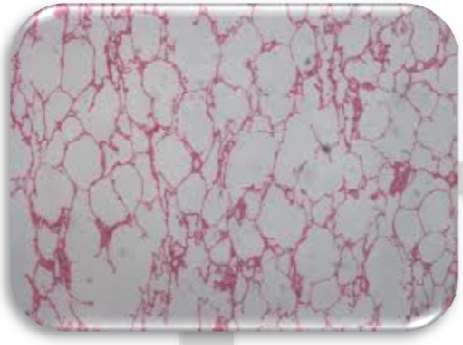


Kidney

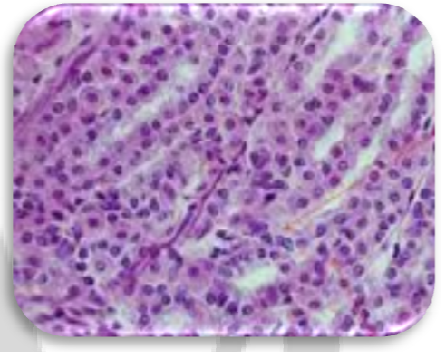
HISTOPATHOLOGICAL FEATURES



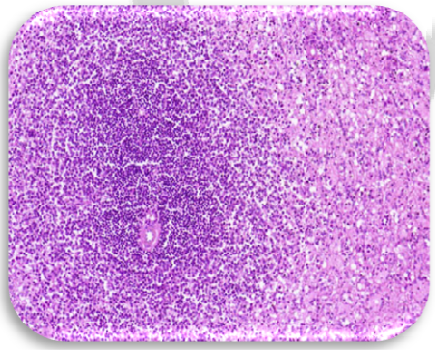
Heart



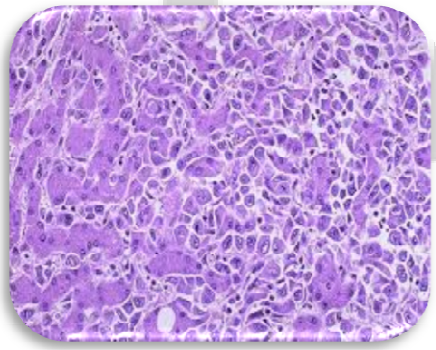
Lungs



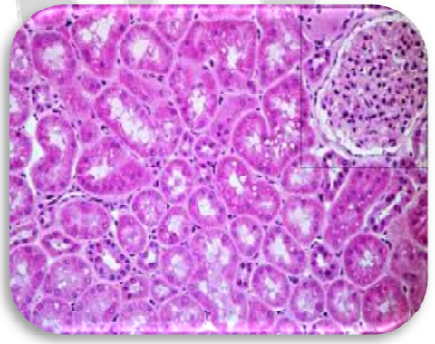
Stomach



Spleen



Liver



Kidney

DISCUSSION

- In developing countries, herbal products prepared from medicinal plants have become famous in healthcare and AHD- 9 is composed of medicinal plant
- There are no scientifically studies for safety and its efficacy of this drug
- The main objectives are to determine the acute toxicity and the sub-acute toxicity of this drug

- Wistar strain rats were used in this study.
- The body weight changes are markers of adverse effects of drugs and if the body weight gain occurred is more than 10% of the initial body weight it will be considered as statistically significant
- The means score of weight of all tested groups were increased at all week and all comparison of means score of weight were statistically ($p=0.00$) significance increase each weekly
- According to the result of this study, this drug was non-toxic

CONCLUSION AND SUGGESTION

- There was no toxic sign at 5000 body weight mg/kg in acute toxicity
- The no-observed adverse-effect level of this drug was found up to 2000 mg/kg body weight for 28 days
- Therefore, the further studies such as experimental animal study and clinical study on healthy volunteers as well as hypertensive patients should be carried out to evaluate the antihypertensive effect of AHD -9

- It is suggested that AHD-9 should be widely used safely and effectively in the treatment of hypertensive disease after these further studies
- Nevertheless, this study could scientifically be proved that the safety of a Myanmar Traditional Medicine formulation used in the treatment of hypertension

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Thank You So Much
For Your Attention!