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EVALUATION OF ACUTE AND SUBCHRONIC TOXICITY OF HYDROETHANOLIC EXTRACT OF *BHARANGYADI* COMPOUND IN RODENTS

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ABSTRACT
This study was aimed at evaluating the safety profile, acute and subchronic toxicity of *Bharangyadi* polyherbal Ayurvedic Compound in rodents, commonly used in the treatment of Bronchial Asthma. Acute Toxicity was evaluated in wister albino rats by administering orally graded doses of extract of *Clerodendrum serratum*, *Hedychium spicatum*, & *Inula racemosa* (*Bharangyadi polyherbal drug*) in the dose of 2000mg, 5000mg, 10g, & 20g/ kg body weight of animal and observed continuously for first 4h and hourly for next 12h, then 8 hourly for next 56hour (72 hour, acute toxicity study). The study had been strictly followed the OECD guidelines. Adult Wister albino rats were divided into 3 groups with 5 animals in each group and a control group, fed with doses of 50mg, 100mg & 150mg/ml twice in a day for 28 days. Effect of drug was evaluated on haematological parameter after every 7 days and histological study was done after sacrificing the animals on 30th day. The median acute toxicity value (LD50) of the extract was found to be infinite and drug was found to be totally non toxic as after ingestion of 20gm no animal found to be dead. Heamatological profile shows no abnormal elevation in level of S.Bilirubin, SGOT, SGPT, S.Urea, & S.Creatinine. The weight of animal rises steadily and appetite increases proportionately with the dose of drug. There was no abnormality found in histopathological assessment. However it was observed that long term use especially at a high dose may prove fatal.
Key Words: *Bharangyadi* Polyherbal compound, Bronchial asthma, Acute toxicity, Subchronic toxicity, Histopathological study.

INTRODUCTION

Bronchial asthma is a chronic inflammatory disease of airways. It may be allergic or non-allergic in origin. Sustain inflammation leads to remodeling of airways and cause diminution of their proper functioning [1-2].

Asthma is said to be only prevented and not cure and this fact underlies its pathophysiological distinctiveness. Asthma proves to be lethal in its acute manifestation as acute onset of severe broncho-constriction may cause grave respiratory distress due to lack of proper oxygen saturation. Asthma whether acute or chronic always associated with permanent damage of normal architecture of airways and thus need a careful and proper management [3-5]. Although contemporary medicines proves beneficial and life saving in bronchial asthma but ultimate dependence on corticosteroids and wide range of toxic side effects forces researcher to think in different dimension and search for some alternative remedy that prove beneficial in preventing as well as cure asthma[6,7].

In an attempt to search some cheap, effective, and fast acting remedy of Bronchial Asthma, some Ayurvedic compound are prepare and planned to be given in areosol form through nebulization. Preclinical study of above said research trial consists of toxicity study of Ayurvedic compound in animal model. *Sati, Bharangi* & *Phuskarmoola* are selected because of their action on *Pranavaha Srotas* (Respiratory tract). *Avapidaka Nasya* and *Pradhmana Nasya* come under the heading of *Sirovirechana Nasya* according to *Susruta*. Whereas according to *Acharya Charaka Avapida Nasya* are of two types *Sodhana & Stambhana*. For *Stambhan Nasya* Acharya advocate use of *Kashaya Skandha* drugs (Ch.Vi.8/144), *Bharangi* belongs to this group so its use for inhalation therapy is based on literary evidences.The route of administration of drug selected consists of both oral and Inhalation route. Whenever an investigator administers a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. These may or may not be harmful to the patients. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD50 (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals). Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds.

MATERIALS & METHODS

Experimental animal

Adult Charles Foster Albino rats (70 $\pm$ 30g) of either sex were obtained from the Animal Research Branch of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were housed in polyvinyl cages and were fed on commercial pellet diet (Amrut, Pranav Agro Industries Ltd, India). They were group housed under standard conditions of temperature (22 $\pm$ 2C^0), relative humidity (60 $\pm$ 5%) and 12:12 light/dark cycle, where lights on at 0700 and off at 1900 h). The saline fed group served as control and one group was treated with a standard drug in each protocol. Before experimentation, the animals were kept on fast for 24 h but water was given *ad libitum* except during experimental test period. During experiments, animals were also observed for any alteration in their general behaviour.

All the experiments and the care of the laboratory animals were according to current ethical guidelines by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

The protocol of this study was approved by Institutional Animal Ethical Committee of Institute of Medical Sciences, BHU- India

Plant Material and Extraction

The plants *Clerodendrum serratum*, *Hedychium spicatum*, & *Inula racemosa* were collected from local market of Varanasi. The identification of the drugs was done by Prof. A.K. Singh, Department of Dravyaguna, S.S.U., Varanasi (Identification number DG/ AKS / 604). Hydroalcoholic Extraction

(Distilled water: Ethanol = 2:1) of drug was carried out by Hot percolation method through soxhlet apparatus. Thereafter extract was dried using rotatory evaporator and dried extract was put to the process of standardization.

Drug Treatment

Standardized extract of Ayurvedic compounds dissolved in distilled water was orally administered as graded doses of 50mg, 100mg, & 150mg/ 100g of bwt of animal twice daily for 28 days for Subchronic toxicity study. Control rats were treated with equal volume of normal saline. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. For Acute toxicity study, standardization extract of *Bharangyadi*– a Polyherbal Ayurvedic compound were administered as escalated doses of 200mg, 500mg, 1g & 2g/ 100g bwt to know the LD 50 value. At each step (i.e. for each dose, starting from lower one) three rodents were used as per **OECD guidelines number 425.**

Toxicity studies: As no literature is available about safety profile of *Bharangyadi* - a polyherbal Ayurvedic drug, it is necessary to perform toxicity studies before its therapeutic use to confirm possible toxic manifestations: following toxicity studies will be performed:

i. **Acute Toxicity study**
ii. **Sub chronic Toxicity study**

Acute Toxicity Study: OECD guidelines (number- 425) was followed for Acute Oral Toxicity study with Up-and –Down-Procedure (UDP). The concept of the up-and-down testing approach was first described by Dixon and Mood [8,9,10].

The method is easiest to apply to materials that produce death within one or two days. According to guideline minimum number of animal should be used for the experiment at each step. The test is divided into two parts: Limit test & Main test.

Limit dose at 2000mg /Kg

Step I

Albino rats of group A were administered 200mg/100 gm bwt of Bharangyadi Hydroethanolic extract dissolved in 1ml of distilled water given orally to 100gm of Albino rat of group- B.

Step II

The rats were observed for 24 hour and when no toxic side effects appears, three more albino rats of Group A with body weight (100 $\pm$ 10g) were administered 2000mg / Kg body weight of Bharangyadi extract and watch for 48 hours. After 48 hours all the rats were survived with no toxic side effect.

Table 1: Body weight variation of treated rats with various doses of the Hydroethanolic extract of *Bharangyadi* during Acute Toxicity Study

Groups	Body weight of rats (before treatment)	Doses of Drug administration	Doses of Drug/ 100g	Body weight of rats (after treatment)		
				24hrs	48hrs	72hrs
A	Ist 55g	0. 55ml	200mg/ ml	70g	75g	85g
	Iind 65g	0. 65ml		80g	85g	90g
	IIIrd 65g	0.65ml		80g	85g	90g
B	Ist 55g	0.55ml	500mg/ml	70g	75g	85g
	Iind 60g	0.60ml		75g	75g	85g
	IIIrd 65g	0.65ml		80g	85g	95g
C	Ist 90g	0.9ml	1g/ml	90g	95g	95g
	Iind 90g	0.9ml		90g	90g	100g
	IIIrd 70g	0.7ml		70g	70g	75g
D	Ist 75g	0.75ml	1g/ml twice within 1/2hrs interval to make total of 2g.	75g	75g	75g
	Iind 70g	0.7ml		70g	70g	70g
	IIIrd 80g	0.8ml		80g	80g	85g

Step IIIrd: According to OECD guidelines if all animal treated with previous low dose survive the dose of the trial drug should be escalated. Thus next step was carried out with Limit dose at 5000mg/ Kg body weight.

Limit Test at 5000 mg/kg

100gm of Albino rat of Group B was treated with 500mg of *Bharangyadi* Extract dissolved in distilled water and observed for 48 hrs. When no toxic side effects were observed even after 48 hrs two more albino rats were administered the above trial dose with their respective body weight and further observed for 48 hrs. All the rats were alive and found healthy after 4 days of treatment.

As after 48hrs the rat survive so two more rats of same group were administered with same dose and observed for any undesired effect.

As even after administration of trial drug in the dose of 5000mg/ Kg body weight, no rat die LD50 of present trial drug is infinite.

To confirm that the present trial drug is non toxic OECD guideline, Page No. 40, was followed. According to the guideline if 2gms oral administration of drug dose not kill the rodent it should be considered as non –toxic.

Step IV: Albino rats of Group D were given 2gm of *Bharangyadi* extract dissolved in 2ml of distilled water in two divided doses at ½ hour interval. Similarly Vth albino rat of Group D was administered 2gms of Shirishyadi Extract dissolved in 4ml of distilled water in four divided doses at 1 hour interval. Both the rats were observed for 48 hours.

Table 2: Effect of Hydroethanolic extract of *Bharangyadi* Extract on various organ weights after 3days of Acute toxicity study

Dose /100g of animals	Lung/ 100gbwt N=3	Liver/ 100gbwt N=3	Stomach/ 100g bwt N=3	Kidney/ 100gbwt N=3	Heart/ 100gbwt N=3	Adrenal/ 100gbwt N=3	Testis/ 100gbwt N=3	Spleen/ 100gbwt N=3
control	948mg ± 1.24	3200mg± 1.76	1078mg ± 2.76	350mg ± 1.22	330mg± 1.55	7.1mg ± 1.28	884mg ± 1.78	281mg ± 0.87
200mg	890mg± 0.87	3000mg ± 2.09	1178mg± 3.21	320mg± 2.56	300mg± 2.54	4.8mg ±0.45	880mg ± 0.77	415mg ± 1.65
500mg	800mg ± 0.56	2980mg ± 1.87	1090mg ± 1.22	298mg± 3.65	280mg ± 2.98	3.5mg ± 0.78	826mg ± 2.45	200mg ± 0.87
1gm	750mg ± 1.09	3800mg ± 2.57	1150mg ± 0.45	250mg± 1.98	268mg± 1.69	8.5mg ± 1.78	798mg ± 2.65	234mg ± 0.67
2gm	900mg± 0.98	4000mg ± 0.99	1200mg ± 1.67	348mg± 1.54	310mg± 1.11	8.6mg± 1.65	850mg ± 2.22	195mg ± 1.43

All the values are Mean ± SDE , where n=3.

Table 3: Acute Toxicity study of Hydroethanolic Etract of *Bharangyadi* in rodents;Death after 7days of treatment:

Group	No. of Albino Rats	Dose of Extract	No. of Dead rats	% Cumulative dead of rats
A	3	200mg/ 100g bwt	0.00	0%
B	3	500mg/100g bwt	0.00	0%
C	3	1g/100g bwt	0.00	0%
D	3	2g/ 100g bwt	0.00	0%

SUB- CHRONIC TOXICITY STUDY[11, 12]

Objectives

1. To establish a "no observable effect level" (NOEL)
2. To characterize dose-response relationships following repeated doses
3. To identify and characterize specific organs affected after repeated administration
4. To predict a reasonable and appropriate dose for chronic exposure studies (maximum tolerated dose or MTD)

Duration: 30 days.

Test System/Animal System: Rodent

Dose Administration : Male and female Wistar albino rats weighing 100 ± 20 g were maintained on standard animal feeds and provided with water *ad libitum*. The animals were weighed and divided into four groups of five animals each. After fasting the rats overnight, the control group received a dose of 0.5 ml of normal saline solution orally once a day for 30 days. The treated groups respectively received the following doses: 50, 100, 150 mg/kg body weight of the hydroalcoholic extract (prepared as in the acute toxicity) orally once daily for 30 days. The animals were then weighed every five days, from the start of the treatment, to note any weight variation .

Parameters

1. Mortality
2. Weight change
3. Signs of toxicity
4. Clinical pathology
5. Pathology and histopathology

Table 4: Variation in body weight of normal and treated rats with various doses of the extract during 30 days of subchronic toxicity study

Dose/Kg bwt	Day 1 Bwt(gms)	Day 5 Bwt(gms)	Day 10 Bwt(mgs)	Day 15 Bwt(mgs)	Day 20 Bwt(mgs)	Day 25 Bwt(mgs)	Day 30 Bwt(mgs)
Control	100	101	105	110	112	113	115
	105	106	108	110	113	115	118
	110	112	114	116	118	120	122
	102	103	105	108	110	111	112
	100	100	105	106	110	110	112
50mg	115	122	125	128	130	135	138
	100	107	110	115	120	122	126
	110	117	122	128	130	132	135
	105	110	115	119	124	126	128
	110	115	120	125	128	130	132
100mg	110	115	120	122	125	130	132
	105	110	115	120	122	124	126
	100	105	109	110	112	115	118
	115	120	125	128	132	135	140
	120	125	130	132	135	136	138
150mg	110	112	115	118	120	122	125
	115	115	117	119	122	125	126
	120	122	124	128	130	133	135
	122	125	126	130	132	135	136
	108	110	112	115	118	120	120

Table 5: Body weight variation of normal and treated rats with various doses of the extract during 30 days of subchronic toxicity study-

Dose/ Kg bwt	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	103± 1.88	104±2.16	107±1.74	110±1.67	112± 1.46	113±1.77	115± 1.98
50mg	108± 2.54	114± 2.69	118 ±2.65	123.4± 2.58	126± 1.93	129±2.28	131.8±2.06
100mg	110± 3.53	115±3.53	119± 3.67	125±3.79	128.2±4.04	130± 3.88	135± 3.70
150mg	113±4.04	116±2.88	116± 2.67	122±2.94	124±2.78	127±2.98	128±3.07

All the values are Mean ± SDE , where n=5.

Table 6: Effect of *Bharangyadi* Compound on Biochemical profile

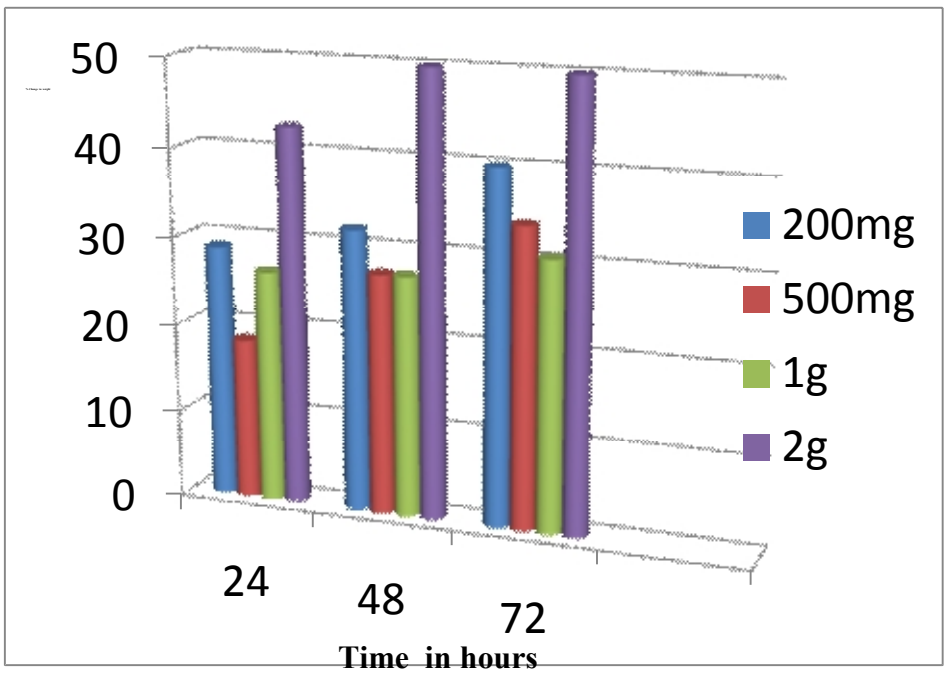
Parameters	After 15 days				After 30 days			
	Control	50mg/ Kgbwt	100mg/ Kgbwt	150mg/ Kbwt	Control	50mg/ Kgbwt	100mg/ Kgbwt	150mg/ Kbwt
B.Urea (mg%)	30.2 ±2.78	37 ± 2.86	40.6± 5.73	48.6± 6.82	46.7± 4.87	40.2 ± 4.89	44.4± 5.43	52.6± 2.54
S.Creatinine (mg%)	0.5± 0.05	0.5 ± 0.05	0.6± 0.01	0.5± 0.08	0.68± 0.09	0.520± 0.02	0.5 ± 0.05	0.68± 0.08
S.Bilirubin(total) (mg%)	0.6± 0.78	0.6± 0.45	0.74± 0.08	0.8± 0.12	0.5± 0.02	0.6± 0.04	0.5± 0.02	0.70± 0.12
S.bilirubin (Direct) (mg%)	0.5 ±0.54	0.5 ± 0.05	0.42 ± 0.06	0.54 ± 0.20	0.52± 0.03	0.48 ± 0.09	0.5± 0.08	0.5± 0.05
SGOT(AST) (IU/L)	150± 4.57	92.7 ± 4.25	100± 7.40	120.7 ± 6.12	150.8± 3.35	120.3± 9.40	107.1± 8.67	138.8± 5.89
SGPT (ALT) (IU/L)	60± 4.38	60.6 ± 2.56	43.4± 5.41	60± 5.90	40.3± 5.36	63.48± 5.29	69.4± 10.63	68± 4.35

All the values are Mean ± SDE , where n=5.

Table 7: Sub-Chronic Toxicity study in albino rats with *Bharangyadi* extract

Groups	No. of Rats	Dose of extract	No. of Dead Rats	% Cumulative dead of Rats
A	5	Control	0	0%
B	5	50mg/ Kgbwt	0	0%
C	5	100mg/Kgbwt	1	20%
D	5	150mg/ Kgbwt	1	20%

Fig. 1, Graph showing the % change in weight of rodent during Acute toxicity study



% Change in body weight of rodents in acute toxicology study.

RESULTS & DISCUSSION

Searching herbs for their medicinal properties and validate their traditional use may prove beneficial by providing a safe and low-cost substitute. Safety and efficacy are two limiting factors in using herbal medicine. Considering the above facts, the safety profile of *Bharangyadi* polyherbal drug was undertaken. *Bharangi* is mainly used in Ayurveda for treating respiratory tract diseases. Bharangi is the most valuable herb to take internally in respiratory ailments and for all fevers in general. As *bharangi* effectively liquefies the mucous, it is salutary in respiratory problems like colds, bronchitis, bronchial asthma and tuberculosis. In such conditions, varied combinations of *bharangi* are recommended. *Hedychium spicatum* is a smallish hardy ginger that grows to around 1 m, with green leaves and large orange and white flowers. Its rhizome is used for treatment of asthma and internal injury.

Powder of rhizome is used as an antiseptic agent and as poultice for various aches and pains. The rootstock is carminative, emmenagogue, expectorant, stimulant, stomachic and tonic. It is useful in the treatment of liver complaints, and is also used in treating fevers, vomiting, diarrhoea, inflammation, pains and snake bite. In preliminary pharmacological studies the drug is found to have a vasodilatory effect on coronary vessels[13, 14], mild hypotensive property and a non-specific antispasmodic effect on smooth muscles. The crude ethanolic extract of rhizomes possesses anti-inflammatory and analgesic activity[15-16]. The anti-inflammatory activity was mainly localized in the hexane fraction from which 1% of pure active constituent was isolated. The analgesic activity was more prominent in the benzene fraction. In Ayurveda *Inula racemosa* is widely used for various disorders, it is mostly used in heart and respiratory disorders. Recent clinical studies also shows that *Phuskarmula* (*Inula racemosa*) is a potent coronary vasodialator agent and also has anti-inflammatory, antianginal and anti ischaemic properties[17].It is also evaluated for hypoglycemic, hypolipidaemic and cardiac activity with highly positive results. It is also acts as antimicrobial agent. Preliminary studies with the ethanolic extract of roots of ***Inula racemosa*** exhibited antiallergic and antiasthmatic properties, the later being more pronounced[18]. Specific studies for bronchodilator properties on isolated trachea were performed and found it a potent bronchodilator. The extract also protected guinea-pigs against various experimental asthma, plant pollen etc. It possessed antihistaminic as well as anti-5-HT activity, suggesting its use in bronchial asthma. Above all these drugs are indicated in bronchial asthma and are being practised in combination successfully since decades although their combination is not given in Ayurvedic texts. Thus it is necessary to evaluate the safety of drug and justify its clinical use.

The acute toxicity study of the extract indicated no changes in the behaviour and in the sensory nervous system responses in the animals. Also no adverse gastrointestinal effects were observed in the albino rats. The LD_{50} calculated for the drug is found to be infinite (according to OECD guidelines) as no rat died even after oral administration of lethal dose of 20g/Kg bwt. Histopathalogically study of visceras showed no microscopic or macroscopic abnormality with no change in colour, no congestion, no necrosis or any other sign of toxicity. Biochemical profile including –Liver function test (LFT), Renal function test (RFT) show no abnormal change substantiated that drug has no renal or hepatotoxicity.

Body weight of albino rats increases propionate with dose of drug but become constant with higher doses. As there was increase in intake of feed with increasing dose of drug, the increase in body weight was not contributed to fluid accumulation in rodents. There was 20% cumulative death in group treated with 150mg of *Bharangyadi* extract during subchornic study whereas 20% in group treated with 100 mg of extract. As there was no morbid sign found prior to death and in addition to this there was no any evidence of toxicity in histopathological studies exclude the possibility of death due to toxic effect of drug.

The result shows that *Bharangyadi* polyherbal compound is totally safe for therapeutic use with infinite LD_{50} value. There were no pathological abnormalities found in histopathological study neither any biochemical abnormalities was identified in scarified rodents after acute toxicity study. In chronic toxicity study, treating rodents with higher doses showed 20% & 20% cumulative death. Absence of any evidence of toxicological sign in histopathological study suggest that death is not due to toxicity of drug and evaluation of safety of drug for long term use should be assess.

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Pathological Changes occurring after Acute Toxicity study

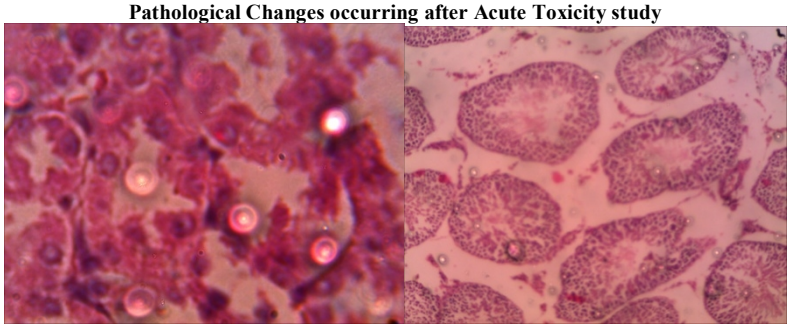

Fig 2: Slide showing the Histopathological changes after acute toxicity study

Fig 3: Feeding albino rat with intubation tube & showing different experiment groups with samples of organ after dissection

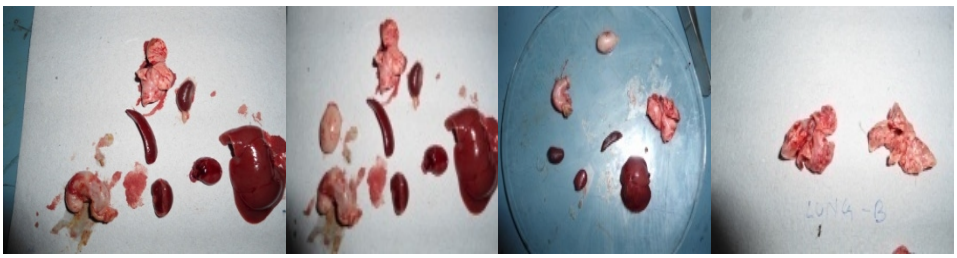

Fig 4: showing different organ taken out after dissection.

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