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A Pharmacological study of *Anupa* and *Jangala Deshastha Shitivaraka* (*Celosia argentea Linn.*). W.S.R. *Mootralkarma*

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ABSTRACT

Pharmacology, the science of drug action, has helped to elucidate many basic physiological and pathological mechanisms in health and disease. Various animal experimental models have been designed to study the effect of drugs on living organisms and isolated tissues. These give an insight about where and how a drug acts, the mode of action of a drug, its effect on various body systems and probable adverse effects before administration of a drug. Therefore, the object of pharmacology is to provide such scientific data in animals as well as humans, which forms the basis of rational therapeutics.

The *Jalamahabhuta* is fundamental base of origin for *kapha dosha and mootra*. These are supposed to have *Asray-Asrayi Sambandha* . It means these are directly proportional to each other. So by using the drug which is having the *mootrala* property *Kapha* may be controlled. Here an effort is made to prove this concept with modern parameters like immunomodulation, anti-inflammatory and antihistaminic activity.

KEYWORDS: *kapha dosha and mootra*, *Asray-Asrayi Sambandha*, immunomodulation, anti-inflammatory and antihistaminic activity

INTRODUCTION

The role of research in ayurveda is not only to elucidate the principles of *ayurveda* but also, to explain them in terms of modern parameters. A number of scholars have carried out research on disease *Tamakashwasa*. This work is a step ahead of those earlier projects. Here an effort is made to substantiate the theory that *Jala* originates from *kapha dosha* and with the help of *mootrala* drug, it may be controlled because *mootra* and *kapha* are suppose to have *Asray-Asrayi Sambandha (Ref.-Aashtang Hriday Sutra.*11/26). It means these are directly proportional to each other; increase in one factor is leads to increase in the other and decrease in one also cause decrease in the other. So by using the drug which is having the *mootrala* property *Kapha* may be controlled; because according to *Ayurveda Kapha* is the main causative factor for the disease *Tamakashwasa*.[1]

Hence the present study was designed to ascertain whether it is possible to obtain experimental data to support the clinical study; and helps to prove the above theory, according to criteria of modern pharmacology too.

AIMS & OBJECTIVES

(1).To asses the test drug for immunomodulation activity.

(2) To evaluate the test drug for anti-inflammatory activity.

(3) To evaluate the test drug for antihistaminic activity.

MATERIALS AND METHODS

TEST DRUG

1. *Anupa Deshastha Shitivaraka (Celosia argentea Linn.)*

2. *Jangala Deshastha Shitivaraka (Celosia argentea Linn.)*

DOSE CALCULATION FOR RAT [2]

The suitable rat dose was calculated by referring the table of Paget and Barnes(1969).

Animal Dose=Human adult dose × Body surface area ratio convertible factor

Human dose – 6g/day (both drug's)

= 6g x 0.018(Conversion factor for rat)

= 0.108 g / 200 g body weight of rat.

= 0.108 x 5 (converted to mg/kg by multiplying with suitable factor 5)

= 0.540 g / kg rat.

= 540mg/kg rat

ANIMAL SELECTION

Wister strain albino rats of either sex weighing 170 to 310 g were used for experiments with the following conditions:

1. The animals were obtained from the Animal House attached to the Pharmacology laboratory I.P.G.T. & R.A.,G.A.U., Jamnagar.
2. They were exposed to natural day and night cycles, with ideal laboratory conditions in terms of ambient temperature and humidity.
3. Temperature during the time of carrying out the experiment was between 20-30 ^{0}C & humidity 50-60%
4. They were fed *ad libitum* with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water.

GROUP'S

1. Group-A (*Anupa Deshastha Shitivaraka*)
2. Group-B (*Jangala Deshastha Shitivaraka*)
3. Group-C (Water control)

ROUTE OF DRUG ADMINISTRATION

Administered according to the body weight of the animals, by oral route with the help of gastric catheter sleeved onto a syringe.

INSTRUMENTS USED

Weighing Scale,Needle,Syringe, Mono pan balance, Rubber Catheter, Mortar & Pestle Refrigerator, Surgical Instruments, Sterilizer Pipette Glass Slides, Watch Glass

CHEMICALS

Triple antigen, Potash alum, Sodium bicarbonate

And normal saline were used for cell mediated immunity test

EXPERIMENTAL MODELS

Experiment

1: Assessment of immunomodulatory activity.

A. Effect on humoral antibody formation.

B. Effect on Cell Mediated Immunity (CMI)

Experiment 2: Anti-inflammatory activity - Carrageenan induced paw oedema

Experiment 3: Antihistaminic activity.

1. IMMUNOMODULATORY ACTIVITY

A. EFFECT ON HUMORAL ANTIBODY FORMATION

Experimental animals - Wister strain Albino Rats.

Sensitizing agent - Sheep Red Blood Cell Corpuscles (SRBC)

PROCEDURE: Sheep blood was collected from the city slaughterhouse in a sterilized bottle containing ACD solution aseptically so that clotting of blood does not take place. It was then subjected for a thorough wash with sterile normal saline and was stored in Alsever's solution in a refrigerator till experimentation. SRBC of 20% V/V concentration was prepared for using as sensitizing agent.

Rats of either sex having body weight in the range between 170-310 g were used in the present study. The animals were allotted into three groups of six (6) animals each. The first group was kept as control and tap water was administered to it. The second group was given *Anupa Shitivaraka* in a dose of 540 mg / kg body wt, third was given *Jangala Shitivaraka* in a dose of 540 mg / kg body weight. The test drug was administered for 09 consecutive days between 9.30 to 10.00 A.M.

On the third day of drug administration the sensitizing agent SRBC (20% v/v) was injected intraperitoneally in a dose of 10 ml/kg body weight. On the 10th day after noting the weight of each animal they were sacrificed by cervical dislocation and the blood was collected in sterile test tubes. Serum was separated from it and complement in it was inactivated by heating it for 30 minutes at 56°C temperature in a serological water bath. Spleen, thymus and lymph nodes were dissected out immediately after the animals were sacrificed and transferred to dish containing normal saline till it is weighed. After noting the weight the organs were transferred to 10% formaldehyde for fixation.

Serial two fold dilutions of the serum in sterile solutions were made in the volume of 0.1 ml in a microtitre plate. Later immediately but carefully 0.1 ml of thrice washed 2% SRBC was added to each well. The trays were covered and placed in a refrigerator overnight. Haemagglutination titre was noted and the reading was converted to log-$_2$ values for easy comparison.

HISTOPATHOLOGICAL STUDIES

Fixation:- Immediately after sacrificing the animals, tissues were excised and extraneous tissue was cleaned of. Pieces of 3-5 mm thickness were cut and transferred to 10% formalin solution and allowed to remain in it till they were taken up for processing.

Tissue Processing:- After thoroughly washing under tap water tissues were placed in 70% alcohol. The tissues were subjected to dehydration, clearing and paraffin infiltration by passing them through 80, 90 and 95% alcohol (2 changes) isopropyl alcohol, acetone (2 changes) chloroform (3 changes), paraffin (2 changes) (3 each). Next the tissues were embedded in paraffin to prepare tissue blocks.

Tissue blocks were fixed to metal object holder after trimming them to suitable sizes.

Section cutting:- The tissue sections (5-mm) were cut with the help of Spencer

type rotating microtone and floated in a water bath between 40 – 45 ^{0}C. They were mounted on clean glass slides with a drop of Mayer's egg albumin, dried on hot plate at about 50 ^{0}C for 30 minutes.

Staining procedure (Haematoxylin Eosin stain):-The sections were stained by serially placing them in xylol, acetone, 95% alcohol, running water, haematoxylin stain, running water again, eosin solution, 95% alcohol (3 charges), acetone (2 charges), xylol (2 charges) and mounted with D.P.X.

Thus prepared sections were scanned in a trinocular research microscope under different magnifications .Changes if any in the cytoarchitecture were noted down.

B. EFFECT OF TEST DRUG ON CELL MEDIATED IMMUNITY

Unlike antibody mediated immune response, which is mediated through the formation of antibody by the plasma cells, in cell mediated immunity T lymphocyte directly reacts with antigen to cause its destruction. Cell mediated immunity is also mediated by release of lymphokines; antibodies and complement are not involved in these reactions. This phenomenon is responsible for the rejection of foreign cells.

The test drug was evaluated to assess its effect on cell mediated immunity.

PROCEDURE:

Rats in the body weight ranging between 130-230 g were used for experiment. The rats were sensitized on first day of drug administration by following solution.

Triple antigen	1 ml
Normal saline	4 ml
Potash alum (10%)	1 ml

pH of the above reagent was maintained between 5.6 - 6.8 using 10% sodium carbonate. On 6th day first initial paw volume of left hind paw was noted and later 0.05 ml of above said solution was injected to it. Volume of the immunological oedema thus produced was measured by volume displacement method (Bhatt et al. 1977), 24 hours and 48 hours later using plethysmograph. Percentage increase over initial value was calculated. To assess the cell mediated immunity values from control group were compared with the data of test drug administered group.

2. ANTI-INFLAMMATORY ACTIVITY

CARRAGEENAN INDUCED PAW OEDEMA

Method of Winter *et al.* (1962) was adopted to screen the anti-inflammatory activity of *Anupa and Jangala Deshastha Shitivaraka* (*Celosia argentea* Linn.) Whole plant powder against carrageenan induced paw oedema in rats. Rats of either sex weighing between 170-310 g were used. Rats were provided with food and tap water up to the start of the experiment. Initially left hind paw volumes up to the tibio-tarsal articulation were recorded by Using a Plethysmograph.

The Plethysmograph employed, consists of 10 ml glass vessel (25 mm x 65 mm) fixed to 2 ml glass syringe through pressure tubing. About 5ml mercury was filled in the syringe and the mercury level was adjusted to zero mark on the micropipette. The space between the zero mark and the fixed mark of the glass vessel was filled with water and few drops of teepol. The initial level of fluid was adjusted and set at zero. The paw was immersed in water exactly up to the tibio-tarsal joint. The increased level

of water in the glass vessel was adjusted to the prefixed mark by releasing the pressure of the connected syringe. The level where water and mercury interface in the micropipette was recorded as paw volume.

PROCEDURE: One hour after drug administration, oedema was produced by injecting 0.1 ml freshly prepared 1% carrageenan in sterile saline solution to the sub-plantar aponeurosis of the left hind limb. The rats were administered with the tap water in the dose of 2 ml / l00g body weight to ensure uniform hydration. This is supposed to minimize the variation in oedema formation. The paw volume is recorded at the interval of lhr, 2hr, 3hr and 6hr.

Results were expressed as percentage increase in paw volume at various intervals of time in comparison to the initial values.

3. ANTIHISTAMINIC ACTIVITY

Effect of test drug on the Guinea pig ileum (in vitro):

Bronchial hyper-responsiveness and inflammatory reaction within the bronchial wall are the important pathological events observed in asthma. These two phenomena are due to release of mast cell mediators such as histamine, prostaglandin and leukotrienes. Because of this reason the test drugs were assessed for anti-histaminic property in isolated guinea pig ileum preparation.[3]

PROCEDURE: This experiment was set-up following standard procedure. A healthy Guinea pig was sacrificed by stunning and severing of neck blood vessels. Abdomen was opened by a mid line incision, ileum was identified, 3-4 cm of it was excised out and placed in a petri dish containing, oxygenated tyrode Solution (NaCl -137, KC1-2.7, $CaCl_2$-l:8, $MgCl_2$-0.1, $NaHC0_3$ -11.9, NaH_2PO_4-0.4 and Glucose-5.55 mm per liter). After placing suitable ligatures the tissue was setup in an isolated organ bath containing tyrode solution, which was oxygenated through, continued passage of 0_2. The tissue was allowed to rest for 30 minutes before eliciting responses to drugs. During resting period the tyrode solution in the organ bath was changed once in every 10 minutes. The tissue response was recorded through frontal writing level system on a smoked drum attached to kymograph (magnification 1: 7 and preload of 500mg). Initially the dose response was recorded with standard spasmogens i.e. to select a dose producing sub maximal response. Recording tissue response to test drugs followed this and its effect on the response elicited with histamine.

4. OBSERVATION & RESULTS

1. Immunomodulatory activity

A. Effect on humoral antibody formation

TABLE – 01

Effect of *anupa and jangala shitivaraka* on body weight of the srbc sensitized albino rats

Groups	Dosage (mg/kg)	Body weight (g)			
		Before treatment	After treatment	Change in weight	% change
Control	Q. S.	221.67 ± 16.42	225.83 ± 15.72	06.83 ± 03.48	----
Group-A	540	241.67 ±	260.80 ±	14.80 ±	116.69 ↑

		12.76	15.14	02.35	
Group-B	540	236.67 ± 20.60	247.25 ± 29.74	06.00 ± 02.45	012.15 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

TABLE – 02

EFFECT OF *ANUPA AND JANGALA SHITIVARAKA* ON WEIGHT OF THYMUS OF THE SRBC SENSITIZED ALBINO RATS

Groups	Dosage (mg/kg)	Thymus weight (g)			
		Absolute weight	% Change	Relative weight (g/100g body wt.)	% Change
Control	Q. S.	00.624 ± 00.100	----	00.286 ± 00.056	---
Group-A	540	00.845 ± 00.091	35.42 ↑	00.326 ± 00.030	13.99 ↑
Group-B	540	00.892 ± 00.076	42.95 ↑	00.376 ± 00.051	31.47 ↑

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

TABLE – 03

Effect of *anupa and jangala shitivaraka* on spleen weight of the srbc sensitized albino rats

Groups	Dosage (mg/kg)	Spleen weight (g)			
		Absolute weight	% Change	Relative weight (g/100g body wt.)	% Change
Control	Q. S.	00.632 ± 00.033	----	00.283 ± 00.012	----
Group-A	540	00.686 ± 00.043	08.54 ↑	00.266 ± 00.021	06.00 ↓
Group-B	540	00.583 ± 00.063	07.75 ↓	00.249 ± 00.048	12.01 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

TABLE – 04

Effect of *anupa and jangala shitivaraka* on anti-body formation in srbc sensitized albino rats

Groups	Dosage (mg/kg)	Anti-body titre value	% Change
Control	Q. S.	05.19 ± 00.53	----
Group-A	540	04.01 ± 00.46	22.74 ↓
Group-B	540	04.67 ± 02.72	10.02 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

HISTOPATHOLOGICAL STUDIES

SPLEEN

The parenchyma of spleen consists of two different kinds of tissue called while pulp and red pulp. While pulp is lymphatic tissue mostly lymphocytes around central arteries. The red pulp consists of venous sinuses filled with blood and cords of spleenic tissue called spleenic (Billroth's) cords. Veins are closely associated with the red pulp. Spleenic cords consist of red blood cells, macrophages lymphocytes, plasma cells and granulocytes. Scanning of sections of spleen from different groups under microscope showed decrease in cellularity in *Anupa desha* sample administered group; where as sections from *Janagala desha* sample did not show any significant change in the cytoarchitecture in comparison to the cytoarchitecture of spleen sections from control group. Fig- 1 to 1e depict photomicrograph of representative sections from different groups.[4]

THYMUS

Scanning of sections of thymus from different groups under microscope was carried out. Cytoarchitecture of thymus sections from drug treated groups were compared with the cytoarchitecture of thymus sections from control group. Thymic sections from *Anupa desha* sample did not show any significant change in the cytoarchitecture. In thymic sections from *Janagala desha* sample administered group decrease in cellularity was observed. Fig- 2 to 2e depict photomicrograph of representative sections from different groups.

LYMPH NODES

Scanning of sections of lymph nodes from different groups under microscope did not reveal any significant change in the cytoarchitecture in lymph node in *Anupa desha* sample treated group in comparison to the cytoarchitecture of lymph node sections from control group. However, the sections from *Janagala desha* sample treated group showed decrease in cellularity. Fig- 3 to 3e depict photomicrograph of representative sections from different groups

B. EFFECT OF TEST DRUG ON CELL MEDIATED IMMUNITY

TABLE – 05

EFFECT OF *ANUPA AND JANGALA SHITIVARAKA* ON IMMUNOLOGICAL OEDEMA IN TRIPLE ANTIGEN SENSITIZED ALBINO RATS

Groups	Dosage (mg/kg)	% Increase in paw volume at different time intrvals			
		24 hrs	% Change	48 hrs	% Change
Control	Q. S.	51.36 ± 10.27	----	35.73 ± 07.00	----
Group-A	540	25.64 ± 02.85*	50.08 ↓	17.42 ± 01.68*	51.25 ↓
Group-B	540	28.46 ± 07.33	44.59 ↓	20.24 ± 03.83	43.35 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease *P <0.05

2. ANTI-INFLAMMATORY ACTIVITY

Table – 06

Anti-inflamatory effect *anupa and jangala shitivaraka* on carrageenin induced paw oedema in albino rats

Groups	Dosage (mg/kg)	% Increase in paw volume	
		3 hrs	% Change
Control	Q. S.	71.57 ± 11.86	----
Group-A	540	64.44 ± 04.76	09.96 ↓
Group-B	540	57.68 ± 03.71	19.41 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

3. ANTIHISTAMINIC ACTIVITY

Effect of test drug on the Guinea pig ileum (in vitro):

ISOLATED TISSUE STUDIES: Effect on isolated guinea pig ileum : both the test drugs failed to produce any effect per se and also did not modify the spasmogenic response of the tissue to histamine up to the dose of 50 µg/ml of bath fluid. The kymographic recordings are provided.

5) DISCUSSION

1) Consolidated statement on pharmacological profile of *Anupa and Jangala Deshastha Shitivaraka*

Table-07

Sr. No	Parameters	Group A Anupa Deshastha Shitivaraka	Group B Jangala Deshastha Shitivaraka
1.	Immunomodulatory activity		
	A) Humoral anti-body formation		
	i) Body weight	NSI	NSE
	ii) Weight of Thymus	NSE	NSI
	iii) Weight of spleen	NSE	NSE
	iv) Anti-body titre (Log_2 value)	NSD	NSE
	B) C.M.I		
	i) 24 hrs	SD	NSD
	ii) 48hrs	SD	NSD
2.	Anti-inflammatory activity (3hrs)	NSD	NSD
3.	Anti-histaminic activity	NE	NE

Abbreviations used in the table are as follows:

Group - A : Anupa Deshastha Shitivaraka;

Group - B : Jangala Deshastha Shitivaraka;

NSE – No significant effect, SI – Significant increase, SD – Significant decrease, NSI – non-significant increase, NSD – Non-significant decrease

2) *Anupa Deshastha Shitivaraka* was found to have better immunosuppressant activity in comparison to *Jangala Deshastha Shitivaraka*. It should be noted the difference between the two samples especially with respect to CMI suppression was not much but due to variation in the data statistical significance with *Jangala Deshastha Shitivaraka* was not observed. Thus apparently though *Anupa Deshastha Shitivaraka* shows better activity profile the difference with respect to efficacy may not be significant.

3) Thus based on the data generated during the study it can be suggested that immunosuppression and weak anti-inflammatory activity in the test drugs is responsible for whatever activity observed.

6) CONCLUSION

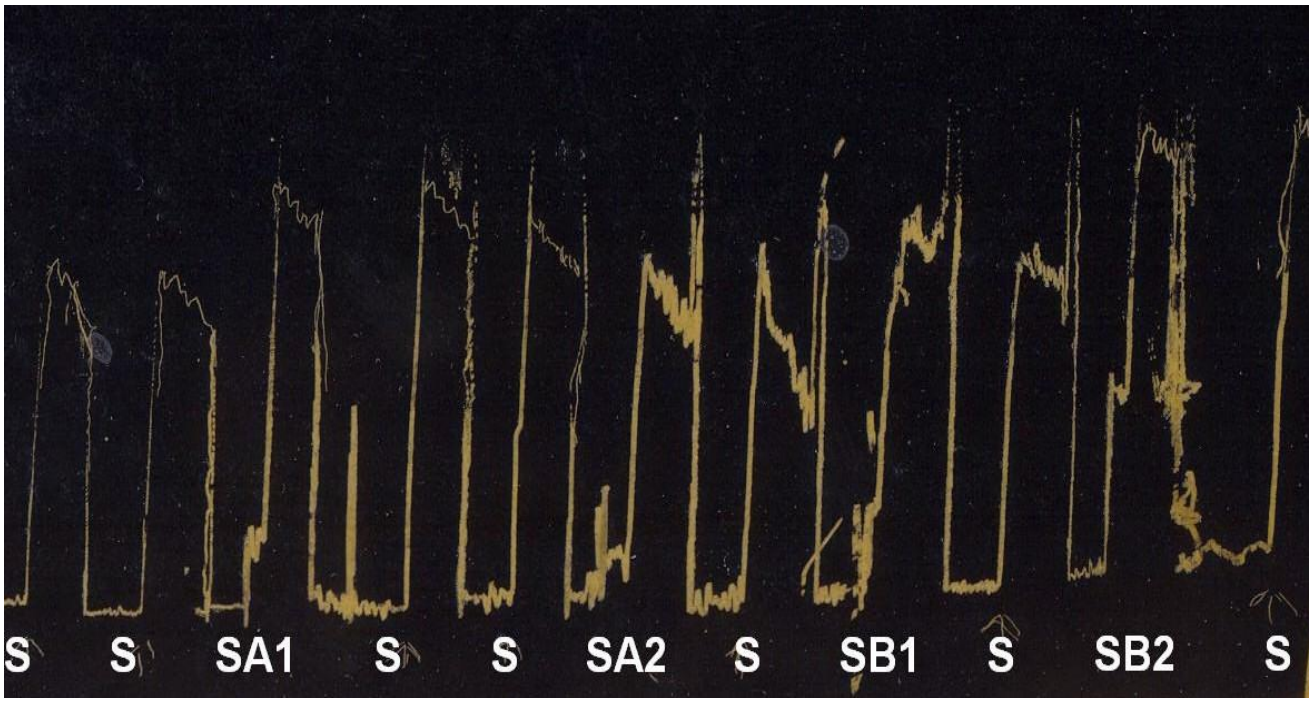
01)The significant CMI suppression effect in *Anupa Deshastha Shitivaraka* and moderate CMI suppression effect in *Jangala Deshastha Shitivaraka* was found. It should be noted the difference between the two samples especially with respect to CMI suppression was not much but due to variation in the data statistical significance with *Jangala Deshastha Shitivaraka* was not observed.

02) The study indicates presence of moderate anti-inflammatory activity in both the samples.

03) Both the samples did not exhibit anti-histaminic activity.

FIG.- 01

Effect of anupa and jangala shitivaraka on isolated guinea pig ileum.



TD: Test dose

S : Sub-maximal response to histamine (500 mg/ml of bath fluid).

A1 : Response to sub-maximal dose of histamine in presence of Anupa Shitivaraka (45 µg/ml of bath fluid)

A2: Response to sub-maximal dose of histamine in presence of Anupa Shitivaraka (90 µg/ml of bath fluid)

B1 : Response to sub-maximal dose of histamine in presence of Jangala Shitivaraka (45 µg/ml of bath fluid)

B2: Response to sub-maximal dose of histamine in presence of Jangala Shitivaraka (90 µg/ml of bath fluid)

(Note: no effect *per se* with the test drug and also on histamine induced contractile response)

ANUP SAMPLE- A, C, E (6.1,6.2,6.3)

JANGAL SAMPLE- B, D, F (6.1,6.2,6.3)

PLATE - 6.1 PHOTOMICROGRAPHS OF SPLEEN

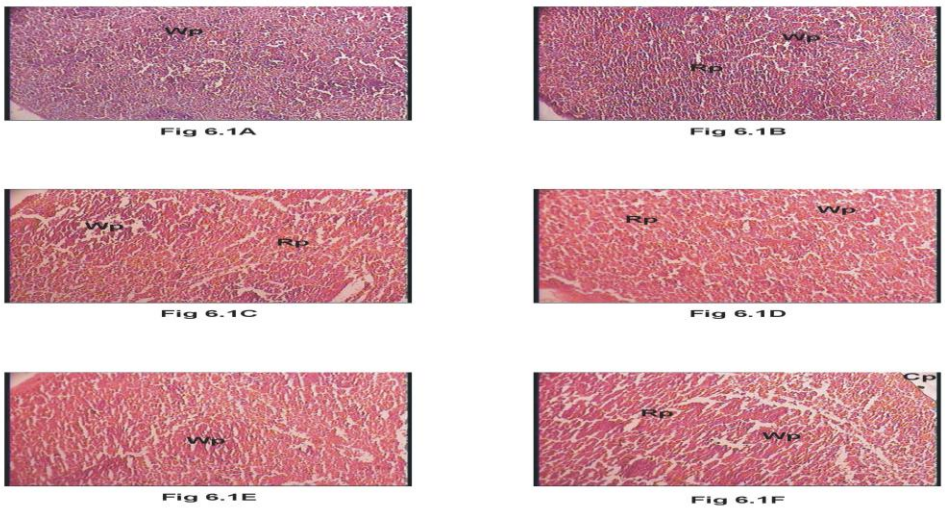


PLATE - 6.2 PHOTOMICROGRAPHS OF THYMAS

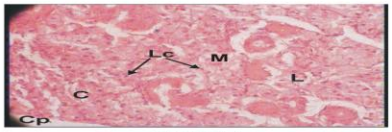


Fig 6.2A

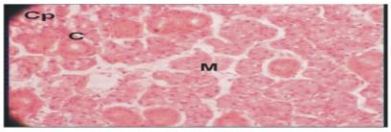


Fig 6.2B

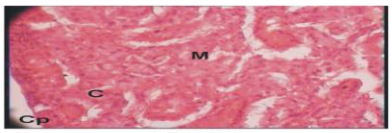


Fig 6.2C

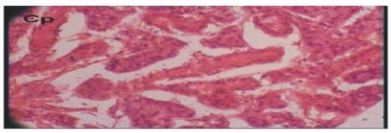


Fig 6.2D

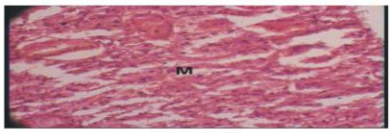


Fig 6.2E

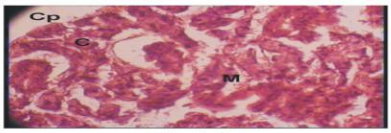


Fig 6.2F

PLATE - 6.3 PHOTOMICROGRAPHS OF LYMPH NODE

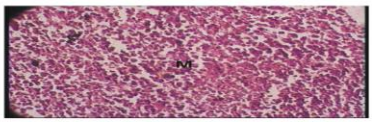


Fig 6.3A

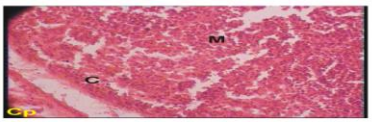


Fig 6.3B

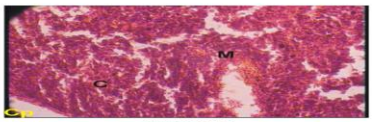


Fig 6.3C

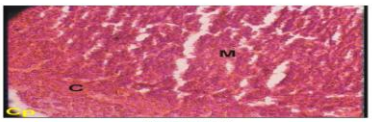


Fig 6.3D

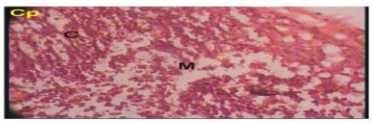


Fig 6.3E

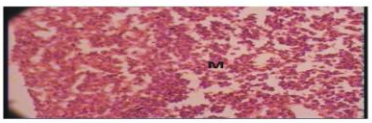


Fig 6.3F

PLATE - 6.4 PHOTOGRAPHS OF MICROTITRE PLATES

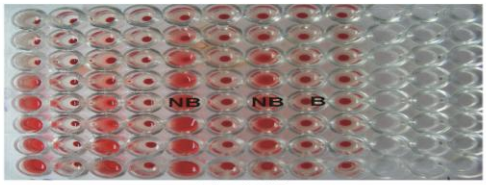


Fig 6.4A

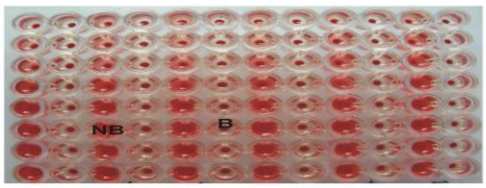


Fig 6.4B

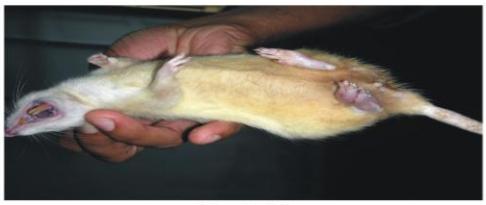


Fig 6.4C

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