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Research Article

PHARMACOLOGICAL EVALUATION OF ANTI-HYPERLIPIDAEMIC ACTIVITY OF *VACHA CHURNA* (*ACORUS CALAMUS* LINN.) AND *MUSTA CHURNA* (*CYPERUS ROTUNDUS* LINN.)

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ABSTRACT

Vacha is identified botanically as *Acorus calmus* Linn belongs to the family Araceae. In Pharmacological study was carried out on Wistar strain albino rats. The activity of the drug was analyzed under the following groups: Group I - Water control, Group II - Cholesterol control, Group III - *Vacha Churna* and Group IV - *Musta Churna*. Parameters like ponderal changes, histopathological study of organs, and biochemical parameters mainly the lipid profile were studied. In present study animals administered with hyperlipidaemic diet have shown increase in body weight in comparison to normal rats, however the observed increase is found to be statistically non-significant. Both the test drugs non-significantly attenuated the body weight, among them the magnitude of decreased bodyweight is more in *Vacha* treated group. In present study administration of hyperlipidaemic diet did not affect the weight if heart, liver and kidney to significant extent in comparison to normal rats. In test drug treated groups also the weight of these organs not affected to significant extent. Administration of *Vacha churna* and *Musta churna* did not affect serum lipid parameters in comparison to cholesterol control rats except on serum HDL cholesterol in which moderate attenuation was observed. The test drugs contrary to the expectation did not significantly lower neither the cholesterol nor the triglyceride level in the serum.

KEYWORDS: Anti-Hyperlipidaemic Activity, *Vacha, Acorus calamus* Linn.), *Musta Cyperus rotundus* L.

INTRODUCTION

The earliest Indian records are Vedas and the world's oldest known pharmacological and therapeutic writing comes from India. From that day lots of references are available regarding the testing of the drugs and foods on the animal for the safety of the mankind. In *Charaka Samhita* numerous references find their way to depict these procedures e.g. in *Siddhi sthana Adhyaya* 6/79 - 80 references to testing on animals can be found. *Sushruta Samhita* has dealt with this by allotting a separate chapter on *Yogya Vidhi* (Su. Su. 9), in which it is said that any procedure which is expected to be performed on human being should undergo trials on animals or other things, having same characteristics and in *Kalpasthana* there is similar discussion dealing with the observations of animal experiments. *Acharya Vagbhata* has also described them in more or less similar manner.

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

AIMS AND OBJECTIVES

1. Evaluation of *Vacha churna* to assess its effect on lipid profile of albino rats for possible use as anti-hyperlipidaemic drug.
2. To compare its efficacy as anti-hyperlipidaemic drug with *Musta churna* which is considered as standard *Ayurved* hypolipidaemic drug.
3. To provide experimental basis to the clinical findings.

MATERIALS AND METHODS

Animals

Wistar strain albino rats of either sex weighing 140 to 240 g were obtained from animal house attached to the Pharmacology Laboratory, I.P.G.T. & R.A., G.A.U., Jamnagar. Animals were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature (22 ± 2ºC) and humidity (50 – 60%). They were fed with Amrut brand rat pellet

feed supplied by Pranav Agro Industries and tap water given *ad libitum*. All procedures and experiments were conducted in day time according to specification of the Indian National Science Academy (INSA). The experiments were carried out after obtaining the permission of Institutional Animal Ethics Committee (Approval number IAEC 09-10/05MD 03).

Dose fixation and schedule

The dose of the formulations was calculated by extrapolating the therapeutic dose to rat dose on the basis of body surface area ratio by referring to the table of Paget and Barnes (1969)[1].

(a)Human dose × conversion factor (0.018) for rat = 'x' / 200 g

(b) 'X' × 5 = 'Y' / kg dose

Human dose – 6g/day

So, by calculating this way, the rat dose of *Vacha churna* is 100mg /kg and *Musta churna* is 550mg /kg.

Preparation of test drug

The drug was crushed into fine powder and made in to fine suspension in distilled water to obtain suitable concentration to administer at a volume of 1ml/200g body wt., while the animals of control group were given only tap water in an equal volume. The drug solutions and tap water were administered with the help of gastric catheter sleeved to syringe.

Parameters studied

1. Ponderal changes

Body weight, Weight of liver, heart and kidney.

2. Histopathology study of liver, heart, kidney and aorta.

3. Biochemical parameters

Serum total cholesterol, serum triglyceride, serum HDL cholesterol, Serum LDL cholesterol, serum VLDL cholesterol, blood sugar, blood urea, serum creatinine, serum total protein, serum albumin, serum globulin, A/G ratio, S.GOT, S.GPT and serum alkaline phosphatase.

Procedures for biochemical parameters estimation

The requisite quantity of serum was fed to the auto analyzer, which was automatically drawn in to the instrument for estimating different parameters. References given in the kit literature mentioning the basis of the methods on which test procedures have been evolved are mentioned along with each of the test mentioned below.

Blood glucose (GOD - POD method, end point)[2], Blood urea (Urease - GLDH - Fixed time, Kinetic, enzymatic method)[3], serum creatinine (Modified Jaffe's reaction)[4], serum total cholesterol (CHOD – PAP, end point)[5], Serum HDL cholesterol (Trinder reaction)[6], serum triglyceride (GOP – PAP method, end point)[7], serum total protein (Biurate Method, End method)[8], serum albumin (BCG Dye method, end point)[9], serum globulin (Serum globulin was calculated from serum protein and serum albumin values), serum alkaline phosphatase (IFCC Method, Kinetic method)[10], SGOT(IFCC method without (Pyridoxal Phosphate)[11], SGPT (IFCC method, kinetic without Pyridoxal Phosphate).[12]

OBSERVATIONS & RESULTS

PONDERAL CHANGES

A) Effect on body weight

Table 1: Effect of *Vacha churna* and *Musta churna* on body weight of hyper hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Body Weight					
		Initial body wt.	Final body wt.	Weight gain	% change	% change of weight	% change
Water control	Q.S.	192.33 ± 02.39	221.33 ± 09.52	32.67 ± 05.90	---	16.63 ± 03.74	---
Cholesterol Control	Q.S.	182.17 ± 17.28	210.00 ± 11.41	27.80 ± 06.78 ↑	14.81 ↓	17.56 ± 05.28	05.60 ↑
Vacha	100	167.00 ± 05.43	187.67 ± 5.35	20.67± 6.9	25.72 ↓	12.85± 4.35	26.82 ↓
Musta	550	173.83 ± 08.19	197.67 ± 7.47	24.17± 4.78	13.15 ↓	14.30± 3.11	18.56 ↓

Data: Mean ± SEM

Data related to the effect of test drugs on body weight of hyper hyperlipidaemic albino rats has been presented in Table - 1. Administration of hyperlipidaemic diet leads to marginal but statistically non-significant increase in body weight in comparison to normal rats. An apparent and statistically non-significant decrease in body weight was observed in both test drug administered groups in comparison to cholesterol control group. This indicates presence of weak to moderate body weight reducing activity in the test drugs.

B) Effect on weight of liver

Table 2 :Effect of *Vacha churna* and *Musta churna* on liver weight of hyper hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Weight of liver (g)		
		Absolute weight (g)	Relative weight (g/100 g body wt)	% change
Water control	Q.S.	8.72 ± 0.66	3.86 ± 0.20	--
Cholesterol Control	Q.S.	6.67 ± 0.35	3.25 ± 0.18	15.80 ↓
Vacha	100	6.28 ± 0.33	3.43 ± 0.10	05.53↑
Musta	550	7.07 ± 0.46	3.57 ± 0.16	10.00↑

Data: Mean ± SEM

The data related to the effect of test drug on liver weight have been shown in Table- 2. Administration of hyperlipidaemic diet leads to marginal but statistically non-significant decrease in weight of liver in comparison to normal rats. Administration of test drugs did not affect the weight of liver to significant extent in comparison to cholesterol control rats.

C) Effect on weight of heart

Table 3: Effect of *Vacha churna* and *Musta churna* on heart weight of hyper hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Weight of Heart (g)		
		Absolute weight (g)	Relative weight (g/100 g body wt)	% change
Water control	Q.S.	0.71±0.03	0.32 ± 0.02	---
Cholesterol Control	Q.S.	0.65±0.03	0.32 ± 0.02	---
Vacha	100	0.63±0.02	0.35 ± 0.02	10.00↑
Musta	550	0.62±0.03	0.31 ± 0.01	---

Data: Mean ± SEM

The data related to the effect of test drug on heart weight have been shown in Table- 3. Administration of hyperlipidaemic diet did not affect the heart weight to significant extent in comparison to normal rats. Administration of *Vacha* leads to a marginal increase in weight of heart which is found to be statistically non-significant. Administration of *Musta* did not affect the weight of the heart to significant extent.

D) Effect on weight of kidney

Table 4: Effect of *Vacha churna* and *Musta churna* on kidney weight of hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Weight of kidney (g)		
		Absolute weight (g)	Relative weight (g/100 g body wt)	% change
Water control	Q.S.	1.43±0.07	0.64±0.02	---
Cholesterol Control	Q.S.	1.27±0.08	0.61±0.01	5.0 ↓
Vacha	100	1.21±0.05	0.66±0.02	8.0 ↑
Musta	550	1.27±0.07	0.64±0.03	5.0 ↑

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

The data related to the effect of test drug on kidney weight have been shown in Table- 4. Administration of hyperlipidaemic diet leads to marginal but statistically non-significant decrease in weight of kidney in comparison to normal rats. Administration of test drugs leads to marginal but statistically non-significant increase in weight of kidney in comparison to cholesterol control rats.

BIOCHEMICAL PARAMETERS

A) Effect on serum cholesterol

Table 5: Effect of *Vacha churna* and *Musta churna* on serum total cholesterol level in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Total cholesterol (mg/dl)	% change
Water control	Q.S.	51.83 ± 03.32	---
Cholesterol Control	Q.S.	77.00 ± 05.54**	48.56 #↑
Vacha	100	71.50 ± 04.90	07.14© ↓
Musta	550	72.50 ± 04.40	05.84 © ↓

Data: Mean ± SEM ↓ - Decrease **P<0.01

(# - In comparison to normal control) (© - In comparison to cholesterol control group)

Data pertaining to effect of test drugs on total cholesterol level have been provided in Table - 5. An apparent and statistically significant increase in total cholesterol level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* and *Musta* lead to statistically non-significant decrease in total cholesterol level in comparison to cholesterol control rats.

B) Effect on serum triglycerides

Table 6: Effect of *Vacha churna* and *Musta churna* on serum triglycerides level in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum triglycerides (mg/dl)	% change
Water control	Q.S.	106.83 ±17.37	---
Cholesterol Control	Q.S.	137.33±20.03	28.55 ↑
Vacha	100	149.67± 24.23	08.98 ↑
Musta	550	131.67±21.64	04.12 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

Data pertaining to effect of test drugs on total tryglyceride level have been provided in Table - 6. An apparent and statistically non-significant increase in serum triglyceride level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* lead to statistically non-significant increase in serum triglyceride level where as administration of *Musta* lead to non-significant decrease in comparison to cholesterol control rats.

C) Effect on serum HDL

Table 7: Effect of *Vacha churna* and *Musta churna* on serum HDL cholesterol level in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum HDL (mg/dl)	% change
Water control	Q.S.	29.67±3.08	
Cholesterol Control	QS	49.17±4.13***	65.72 ↑
Vacha	100	39.83±4.37	18.99↓
Musta	550	43.17±0.95	12.20↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease ***P<0.01

Data pertaining to effect of test drugs on HDL cholesterol level have been provided in Table - 7. An apparent and statistically significant increase in HDL cholesterol level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* and *Musta* lead to statistically non-significant decrease in HDL cholesterol level in comparison to cholesterol control rats.

D) Effect on serum VLDL

Table 8: Effect of *Vacha churna* and *Musta churna* on serum VLDL cholesterol level in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum VLDL cholesterol level (mg/dl)	% change
Water control	Q.S.	21.37 ± 03.47	--
Cholesterol Control	Q.S.	31.13 ± 04.82	45.67 ↑
Vacha	100	29.93 ± 04.84	03.72 ↓
Musta	550	26.33 ± 04.33	15.41 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

Data pertaining to effect of test drugs on VLDL level have been provided in Table - 8. An apparent and statistically non-significant increase in VLDL level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* and *Musta* lead to statistically non-significant decrease in VLDL level in comparison to cholesterol control rats.

E) Effect on serum glucose

Table 9: Effect of *Vacha churna* and *Musta churna* on serum glucose level in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum glucose (mg/dl)	% change
Water control	Q.S.	136.17 ± 04.14	
Cholesterol Control	Q.S.	094.33 ± 04.29***	30.72 ↓
Vacha	100	121.83 ± 07.76**	29.15 ↑
Musta	550	101.33 ± 10.01	07.42 ↑

Data: Mean ± SEM ↑ - Increase ↓ - Decrease ***P<0.001 **P<0.05

Data pertaining to effect of test drugs on serum glucose level have been provided in Table - 9. An apparent and statistically significant decrease in serum glucose level was observed in cholesterol control rats in comparison

to normal rats. Administration of *Vacha* lead to statistically significant increase in serum glucose level in comparison to cholesterol control rats where as *Musta* lead to non-significant increase in serum glucose level.

F) Effect on serum proteins:

Table 10: Effect of *Vacha churna* and *Musta churna* on serum protein level in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum proteins (mg/dl)	% change
Water control	Q.S.	7.18 ± 0.27	
Cholesterol Control	Q.S.	8.40 ± 0.24**	17.00 ↑
Vacha	100	7.17 ± 0.63	14.64 ↓
Musta	550	7.30 ± 0.56	13.09 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease **P<0.01

Data pertaining to effect of test drugs on serum total protein level have been provided in Table - 10. An apparent and statistically significant increase in serum total protein level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* and *Musta* lead to statistically non-significant decrease in serum total protein level in comparison to cholesterol control rats.

G) Effect on serum albumin

Table 11: Effect of *Vacha churna* and *Musta churna* at two dose level on serum albumin level in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum albumin(mg/dl)	% change
Water control	Q.S.	3.78 ± 0.23	---
Cholesterol Control	Q.S.	4.37 ± 0.12*	15.61 ↑
Vacha	100	4.12 ± 0.11	05.72 ↓
Musta	550	4.17 ± 0.11	04.57 ↓

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

Data pertaining to effect of test drugs on serum albumin level have been provided in Table - 11. An apparent and statistically significant increase in serum albumin level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* and *Musta* lead to statistically non-significant decrease in serum albumin level in comparison to cholesterol control rats.

H) Effect on serum globulin

Table 12: Effect of *Vacha churna* and *Musta churna* on serum globulin level in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum globulin (mg/dl)	% change
Water control	Q.S.	3.40 ± 0.14	--
Cholesterol Control	Q.S.	4.03 ± 0.16*	18.53↑
Vacha	100	3.77 ± 0.26	06.45↓
Musta	550	3.57 ± 0.19	11.41↓

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

Data pertaining to effect of test drugs on serum globulin level have been provided in Table - 12. An apparent and statistically significant increase in serum globulin level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* and *Musta* lead to statistically non-significant decrease in serum globulin level in comparison to cholesterol control rats.

I) Effect on serum A/G ratio:

Table 13: Effect of *Vacha churna* and *Musta churna* serum A/G ratio in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum A/G ratio (mg/dl)
Water control	Q.S.	1.10 ± 0.08
Cholesterol Control	Q.S.	1.12 ± 0.05
Vacha	100	1.13 ± 0.08
Musta	550	1.22 ± 0.08

Data: Mean ± SEM ↑ - Increase

Data pertaining to effect of test drugs on serum A:G ratio have been provided in Table - 13. Hyperlipidaemic diet did not affect the A/G ratio. Administration of test drugs did not affect the serum A:G ratio to significant extent.

J) Effect on blood urea

Table 14: Effect of *Vacha churna* and *Musta churna* at two dose level on serum urea in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum urea level (mg/dl)	% change
Water control	Q.S.	35.33 ± 2.51	---
Cholesterol Control	Q.S.	47.33 ± 2.94**	33.96 ↑
Vacha	100	36.83 ± 2.74*	22.18 ↓
Musta	550	33.33 ± 1.45***	29.57 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease ***P<0.001 **P<0.01 **P<0.05

Data pertaining to effect of test drugs on serum urea level have been provided in Table - 14. An apparent and statistically significant increase in serum urea level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* and *Musta* significantly attenuated the hyperlipidaemic diet induced elevation of serum urea level in comparison to cholesterol control rats.

K) Effect on serum creatinine

Table 15: Effect of *Vacha churna* and *Musta churna* on serum creatinine in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum creatinine level (mg/dl)	% change
Water control	Q.S.	0.57 ± 0.02	---
Cholesterol Control	Q.S.	0.80 ± 0.03***	40.35 ↑
Vacha	100	0.72 ± 0.05	10.00↓
Musta	550	0.68 ± 0.03*	15.00↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease ***P<0.001 *P<0.05

Data pertaining to effect of test drugs on serum creatinine level have been provided in Table - 15. An apparent and statistically significant increase in serum creatinine level was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* decreased it non-significantly whereas *Musta* decreased it to significant extent in comparison to cholesterol control rats.

L) Effect on S.GOT activity

Table 16: Effect of *Vacha churna* and *Musta churna* on S.GOT activity in hyperlipidaemic albino rats

Group	Dose (mg/kg)	S.GOT (IU)	% change
Water control	Q.S.	175.00 ± 12.06	---
Cholesterol Control	Q.S.	149.40 ± 18.88	14.62 ↓
Vacha	100	190.17 ± 24.27	27.28 ↑
Musta	550	143.00 ± 18.80	04.28 ↓

Data: Mean ± SEM ↑ - Increase ↓ - Decrease

Data pertaining to effect of test drugs on S.GOT activity have been provided in Table - 16. An apparent and statistically non-significant decrease in S.GOT activity was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* increased it to non-significant extent while in *Musta* administered group the values were similar to the values observed in cholesterol control rats.

M) Effect on S.GPT

Table 17: Effect of *Vacha churna* and *Musta churna* on S.G.P.T. activity in hyperlipidaemic albino rats

Group	Dose (mg/kg)	S.G.P.T. activity (IU)	% change
Water control	Q.S.	72.17 ± 05.16	---
Cholesterol Control	Q.S.	128.5 ± 18.78*	78.05↑
Vacha	100	81.50 ± 14.02*	36.57↓
Musta	550	69.67 ± 13.12*	45.78↓

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

Data pertaining to effect of test drugs on S.GPT activity have been given in Table - 17. An apparent and statistically significant increase in S.GPT activity was observed in cholesterol control rats in comparison to normal

rats. Administration of *Vacha* and *Musta* significantly attenuated the observed S.GPT activity elevation in comparison to cholesterol control rats.

N) Effect on serum alkaline phosphatase activity

Table 18: Effect of *Vacha churna* and *Musta churna* on serum ALP activity in hyperlipidaemic albino rats

Groups	Dose (mg/kg)	Serum ALP activity (IU)	% change
Water control	Q.S.	346.83 ± 39.61	----
Cholesterol Control	Q.S.	544.00 ±31.50*	56.84 ↑
Vacha	100	688.00 ± 49.57	26.47 ↑
Musta	550	562.40 ± 67.78	03.30 ↑

Data: Mean ± SEM ↑ - Increase *P<0.05

Data pertaining to effect of test drugs on ALP activity have been presented in Table - 18. An apparent and statistically significant increase in ALP activity was observed in cholesterol control rats in comparison to normal rats. Administration of *Vacha* caused a moderate but statistically non-significant elevation of ALP activity in comparison to cholesterol control rats. In *Musta* administered group only a non-significant marginal elevation was observed in comparison to cholesterol control rats.

EFFECT ON CYTOARCHITECTURE OF DIFFERENT ORGANS

1. **Liver:** Examination of section of liver from hyperlipidaemic diet given rats did not reveal any remarkable change in the cytoarchitecture in comparison to sections from the normal control rats. In *Vacha* and *Musta* administered hyperlipidaemic diet given rats also normal cytoarchitecture was observed. Plate – 5.1 & 5.2 contains photomicrographs of representative sections from different groups.
2. **Kidney:** Examination of kidney sections obtained from hyperlipidaemic diet given rats showed mild fatty changes in few rats and normal cytoarchitecture in the remaining rats. In kidney sections obtained from *Vacha* and *Musta* administered and hyperlipidaemic diet given rats normal cytoarchitecture was observed. Plate – 5.3 & 5.4 contains photomicrographs of representative sections from different groups.
3. **Heart:** Examination of section of heart from hyperlipidaemic diet given rats did not reveal any remarkable change in the cytoarchitecture in comparison to sections from the normal control rats. In *Vacha* and *Musta* administered hyperlipidaemic diet given rats also normal cytoarchitecture was observed. Plate – 5.5 & 5.6 contains photomicrographs of representative sections from different groups.
4. **Aorta:** Examination of sections of aorta from hyperlipidaemic diet given rats did not reveal any remarkable change in the cytoarchitecture in comparison to sections from the normal control rats. In *Vacha* and *Musta* administered hyperlipidaemic diet given rats also normal cytoarchitecture was observed. Plate – 5.7 & 5.8 contains photomicrographs of representative sections from different groups.

CONCLUSION

The both drugs though were not very effective in lowering the hyperlipidaemic diet induced elevated lipid levels are quite effective in attenuating the hyperlipidaemia induced functional disorders. In test drug treated groups also the weight of these organs not affected to significant extent. Administration of *Vacha churna* and *Musta churna* did not affect serum lipid parameters in comparison to cholesterol control rats except on serum HDL cholesterol in which moderate attenuation was observed. The test drugs contrary to the expectation did not significantly lower neither the cholesterol nor the triglyceride level in the serum.

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Plate - 5.1
Photomicrographs of liver

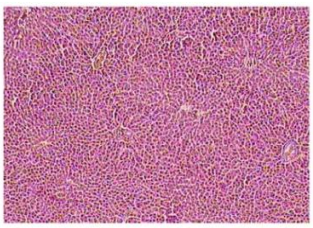

Fig. 5.1a

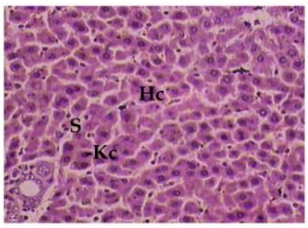

Fig. 5.1b

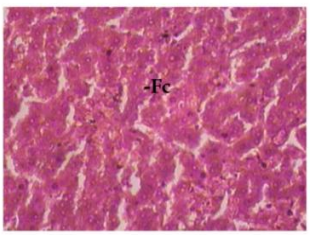

Fig. 5.1c

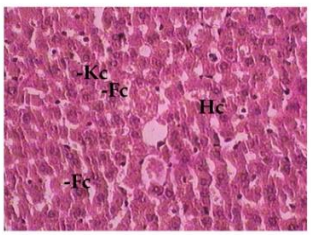

Fig. 5.1d

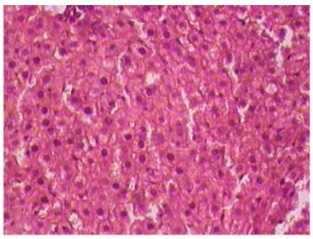

Fig. 5.1e

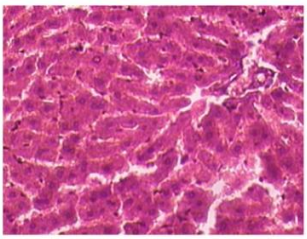

Fig. 5.1f

Plate - 5.2
Photomicrographs of liver

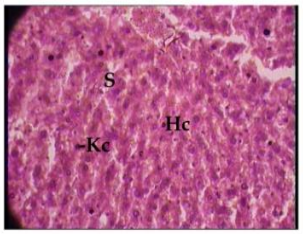

Fig. 5.2a

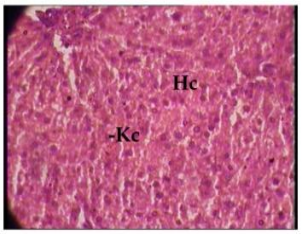

Fig. 5.2b

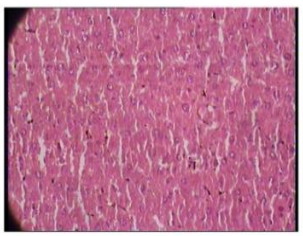

Fig. 5.2c

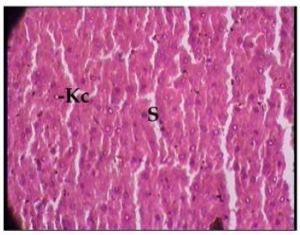

Fig. 5.2d

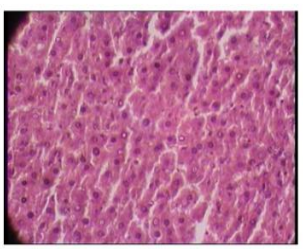

Fig. 5.2e

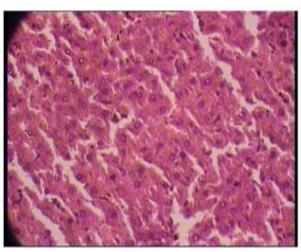

Fig. 5.2f

Plate - 5.3
Photomicrographs of kidney

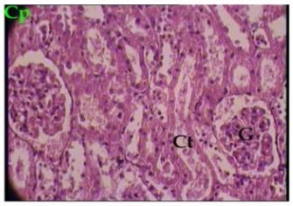

Fig. 5.3a

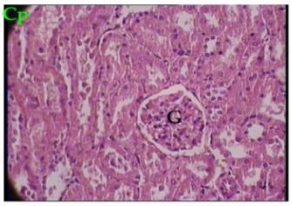

Fig. 5.3b

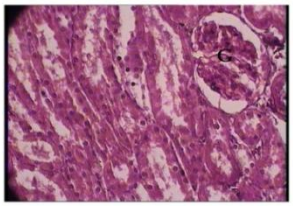

Fig. 5.3c

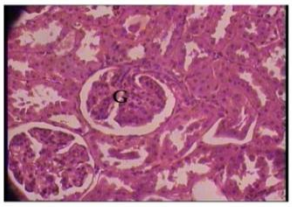

Fig. 5.3d

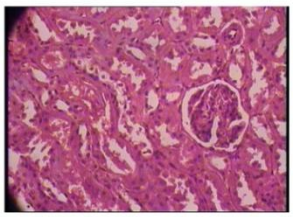
Fig. 5.3e

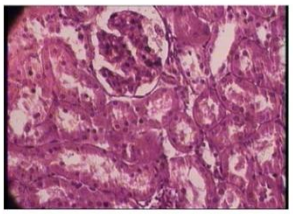
Fig. 5.3f

Plate - 5.4
Photomicrographs of kidney

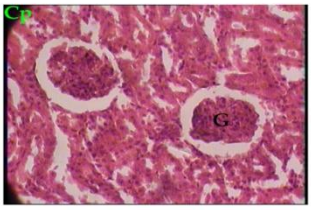

Fig. 5.4a

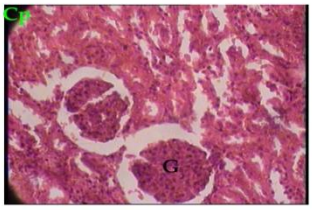

Fig. 5.4b

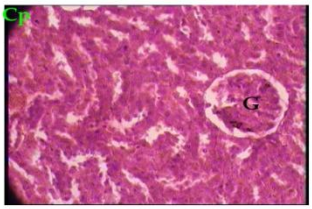

Fig. 5.4c

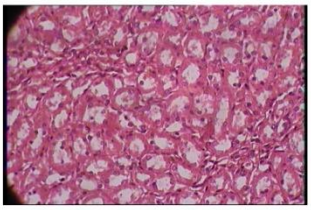
Fig. 5.4d

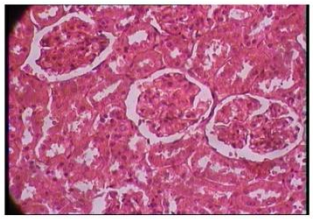
Fig. 5.4e

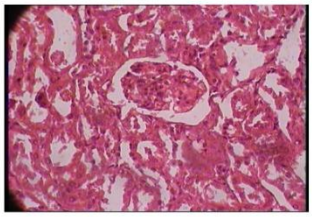
Fig. 5.4f

Plate - 5.5
Photomicrographs of heart

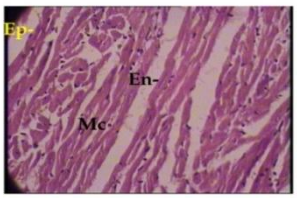

Fig. 5.5a

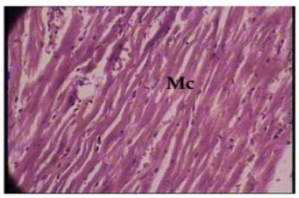

Fig. 5.5b

Fig. 5.5c

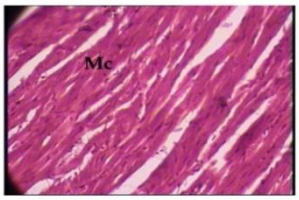

Fig. 5.5d

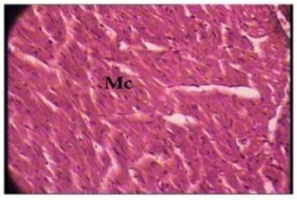

Fig. 5.5e

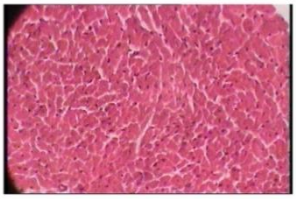
Fig. 5.5f

Plate - 5.6
Photomicrographs of heart

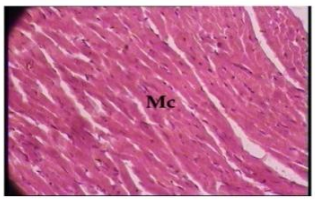

Fig. 5.6a

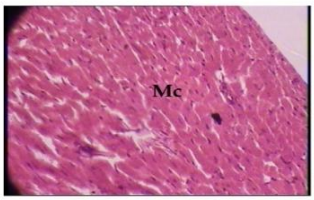

Fig. 5.6b

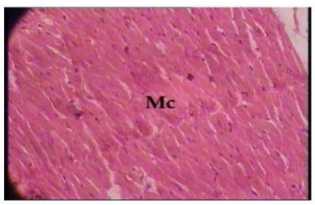

Fig. 5.6c

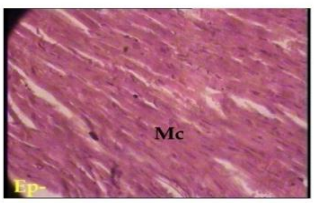

Fig. 5.6d

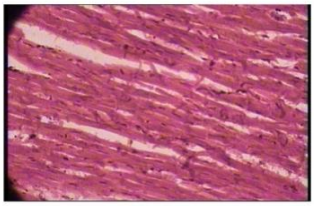
Fig. 5.6e

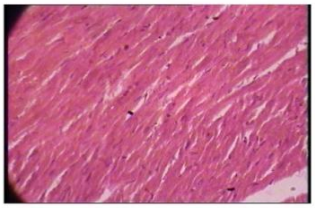
Fig. 5.6f

Plate - 5.7
Photomicrographs of Aorta

Fig. 5.7a

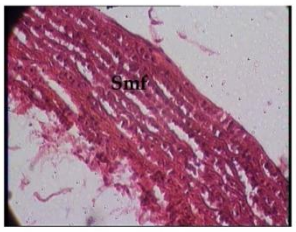

Fig. 5.7b

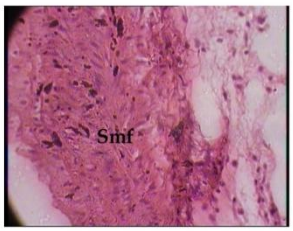

Fig. 5.7c

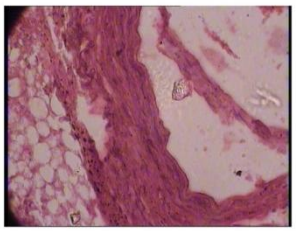

Fig. 5.7d

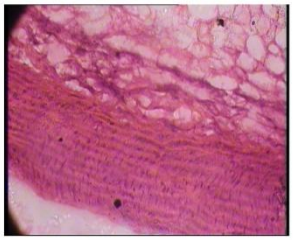

Fig. 5.7e

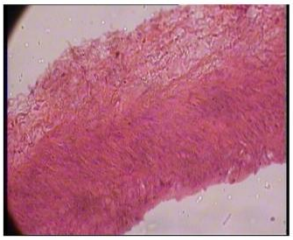

Fig. 5.7f

Plate - 5.8
Photomicrographs of Aorta

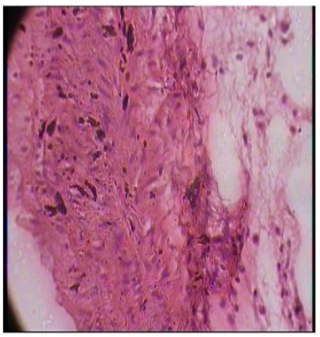

Fig. 5.8a

Fig. 5.8b

Fig. 5.8c

Fig. 5.8d