

(PROFORMA FOR SUBMISSION OF FINAL REPORT OF RESEARCH PROJECTS)**Part - I: General Information****800 Project Code:**

8001 Institute Project Code No. :

8002 ICAR Project Code No. : **IXXO5433****801 Name of the Institute and Division:**

8011 Name & Address of the Institute: Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Hebbal, Bangalore-24

8012 Name of the Division/Section: Epidemiology

8013 Location of the Project: Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Hebbal, Bangalore-24

802 Project Title: “COMPARATIVE VIRULENCE OF *TRYPANOSOMA EVANSI* INFECTION IN RODENT MODEL”**803 Priority Area:**8031 Research Approach: AppliedRes./ **BasicRes.**/ Process Dev/ Tech. transfer
01 02 03 04**804 Specific Area: Experimental research****805 Duration of the Project:**

8051 Date of Start: September 2008

8052 Date of Completion: August 2010

806 Total Cost/Expenditure Incurred: Rs.12,00,000/-

(Give reasons for variation, if any, from the original estimated cost)

807 Executive Summary:

Successful establishment of Trypanosome infections in Wistar albino rats with buffalo and canine isolates of *Trypanosoma evansi*. Collection of blood, tissues like liver, heart, kidney, adrenal, lung, spleen, abdominal muscles after sacrificing rats sequentially from day one to day seven post infection. Histopathological changes were observed in lung, heart, spleen and testis in buffalo isolate but lung, liver, kidney and spleen showed changes in lion and leopard isolate. There is variation in the pathogenicity of various isolates of *Trypanosoma evansi*.

208 Key Words: Wistar albino rats-Pathogenicity – Trypanosomes (buffalo, canine, lion and leopard isolate)

Part - II Investigator Profile

810 Principal Investigator:

8101 Name : Dr. P. Krishnamoorthy
8102 Designation : Scientist
8103 Division/Section : Epidemiology
8104 Location : PD_ADMAS, Bangalore
8105 Institute Address : PD_ADMAS, Bangalore

811 Co-investigator:

8111 Name : Dr. P.P. Sengupta
112 Designation : Senior Scientist
8113 Division/Section : Parasitology
8114 Location : PD_ADMAS, Bangalore
8115 Institute Address : PD_ADMAS, Bangalore

812 Co-investigator:

8121 Name : Dr. M.R. Gajendragad
122 Designation : Principal Scientist
8123 Division/Section : Epidemiology
8124 Location : PD_ADMAS, Bangalore
8125 Institute Address : PD_ADMAS, Bangalore

813 Co-investigator:

8131 Name : Dr. K. Prabhudas
8132 Designation : Project Director
8133 Division/Section :
8134 Location : PD_ADMAS, Bangalore
8135 Institute Address : PD_ADMAS, Bangalore

Part - III: Technical Details

820 Introduction and objectives:

8201 Project Objectives:

1. To know the sequential pathology of different isolates of *Trypanosoma evansi* in Wistar albino rats.
2. To find the various biochemical, histopathological and antioxidant status during different isolates of *Trypanosoma evansi* infection.

8202 Background Information and Importance of the Project:

Trypanosoma evansi is a common parasite in the tropics that causes a serious and economically important disease in domestic animals. The acute form of the disease is

characterized by progressive anaemia, high fever, anorexia, marked depression, dullness, loss of condition and in some cases, death. The chronic form which is more common shows relapsing parasitaemia with or without pyrexia, emaciation, oedema of the abdomen and legs, abortion and death in some animals (Raisinghani *et al.*, 1980; Haroun *et al.*, 2000). More recently, a case of *T. evansi* trypanosomosis in humans was recorded for the first time in India (Joshi *et al.*, 2005). Various isolates of *Trypanosoma evansi* is available in the parasitology laboratory of PD-ADMAS, Bangalore. The virulence of various isolates of *Trypanosoma evansi* will be studied in rodent model in the present study.

821 Project Technical Profile:

8211 Technical Programme:

(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environments, etc.)

1. Induction of experimental infection of Wistar albino rats with different isolates of *Trypanosoma evansi* parasite and one control with each group consisting of six rats. Four isolates of trypanosomes from buffalo, canine, lion and leopard was inoculated intraperitoneally of 0.2ml containing 1×10^5 trypanosomes per ml. The rats were observed for clinical signs during the experimental period.
2. Sequential collection of blood, serum and tissues (liver, lung, spleen, heart, kidney, abdominal muscle, testis and adrenal) samples were done during the course of infection in rat from day one to day seven post infection.
3. Counting of trypanosomes in blood using haemocytometer method, to know the pattern of infection between the different isolates of *Trypanosoma evansi*.
4. Histopathological studies were carried out.
5. Data compilation, analysis and report writing.

8212 Total Man-months Involvement of Component Project Workers:

No.	Nature of work	Activity	Preceding activity	Time (months)	Scientist involved	Time utilization (months)
1	Experimental infection of rats with different isolates of <i>Trypanosoma</i> parasite	A	-	6	1,2	3,3
2	Blood and clinical sample collection from rats	B	A	4	1,2	2,2
3	Histopathology and antioxidant estimation	C	B	6	1,3	3,3
4	Analysis of data	D	C	4	1,3	2,2
5	Compilation of results and report writing	E	D	4	1,2,3,4,	1,1,1,1
	Total			24		24

No	Name	Designation	Time to be spent	Work to be done
1	Dr. Krishnamoorthy, P.,	Scientist	40%	Animal experiments, Sample collection & analysis, Data analysis, interpretation of results and report writing
2	Dr. P. P. Sengupta	Senior Scientist	25%	Establishment of Trypanosoma infection of different isolates in rats and monitoring of infection, report writing
3	Dr. M.R. Gajendragad	Principal Scientist	25%	Histopathological studies and report writing
4	Dr. K. Prabhudas	Project Director	10%	Guidance and advisory capacity

822 Final Report on the Project:

Detailed report containing all relevant data with a summary of results (Not exceeding 2-5 pages)

1. Successful establishment of Trypanosome infections in Wistar albino rats with buffalo, canine, lion and leopard isolates of *Trypanosoma evansi*.

2. Collection of blood, tissues like liver, heart, kidney, adrenal, lung, spleen, abdominal muscles after sacrificing rats sequentially from day one to day seven post infection. The serum biochemical parameters were estimated but there was no much difference in the parameters except glucose and urea levels.

3. From 2 DPI trypanosomes were found in circulating blood and progressive increase in count of trypanosome per ml up to 6 DPI. One rat died on 7 DPI due to severity of infection. Serum glucose was decreased and increased urea from 2 DPI in rats infected with buffalo isolate. On gross examination no changes observed. Histopathological changes were observed in lung, heart, spleen and testis in buffalo isolate. Heart, abdominal muscle, pancreas and adrenal showed no observable changes. Lung showed progressive damage from 2 DPI with focal infiltration of mononuclear inflammatory cells, damage to the alveoli. Liver showed vacuolar degeneration with loss of hepatocytes from 3 DPI. Kidney showed damage to tubules and glomeruli with mononuclear cell infiltration progressively from 2 DPI. Spleen showed damage to lymphocytes on 5 DPI.

4. The lion isolate from 3 DPI trypanosomes were found in circulating blood and progressive increase in count of trypanosome per ml up to 7 DPI. Serum glucose was decreased from 3 DPI and increased urea from 2 DPI. On gross examination no changes observed. Histopathological changes were observed in lung, liver, kidney and spleen in lion and leopard isolate. Lung showed progressive damage from 2 DPI, atelectasis and infiltration of inflammatory cells, damage the alveoli. Liver showed mononuclear cell infiltration and vacuolar degeneration from 3 DPI. Kidney showed damage to tubules and glomeruli with mononuclear cell infiltration progressively from 2 DPI. Spleen showed loss of lymphocytes and reticulum cell hyperplasia on 7 DPI. There is no significant difference in other serum biochemical parameters and antioxidant status in rats.

5. On the basis of pathogenicity, the buffalo isolate was found to be severe when compare to other isolates. The various isolates varied in their predilection of organs affected and the tissue damage caused. Further systematic studies are required with some more isolates is required.

Table1. Serum glucose and urea (g/L) levels in rats inoculated with various isolates of *T. evansi*

Days	Glucose		Urea	
	Buffalo isolate	Lion isolate	Buffalo isolate	Lion isolate
control	112	77.7	18	17.31
1 DPI	119	83.25	27	17.07
2 DPI	79	65.76	48	49.02
3 DPI	65	44.58	44	50.04
4 DPI	45	40.64	51	61.7
5 DPI	38	35.62	65	70.97
6 DPI	35	32.45	71	72.68
7 DPI	Died	31.23	Died	76.58

8221 Achievements in Terms of Targets Fixed for Each Activity:

8222 Questions - Answered:

What is the pattern of virulence of *Trypanosoma* isolated from different species of animals in rats?

8223 Process/Product/Technology/Developed: Nil

8224 Practical Utility:

(Not more than 150 words)

The project compared the virulence pattern of various isolates of *Trypanosoma evansi* in Wistar albino rats. The isolates vary in their pathogenicity in the rats but buffalo isolate found to be more virulence when compared to other isolates. The virulence pattern of the available isolates were studied in this project work.

8225 Constraints, if any: Nil

823 Publications and Material Development:

(One copy each to be supplied with this Proforma)

8231 Research Papers:

1. **Krishnamoorthy, P.,** Sengupta, P.P, Balachandran, C, Gajendragad, M.R. and K. Prabhudas. (2009). Pathology of experimental *Trypanosoma evansi* (Buffalo isolate) infection in Wistar Albino Rat. In: *International symposium on "Philosophy of disease diagnosis through morphological to biomolecular approaches" and core theme of diagnostic*

- pathology*”, 28-30 October 2009, College of Veterinary sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India. **pp**:194.
2. **Krishnamoorthy, P.**, Sengupta, P.P., Gajendragad, M.R. and K. Prabhudas (2010). Pathology of experimental *Trypanosoma evansi* (canine isolate) infection in Wistar albino rat. In: *National symposium on “Recent trend in diagnosis and pathology of emerging and re-emerging disease of livestock and poultry”*. 25-27 November, 2010, College of Veterinary Science, Guwahati, Assam, India. **pp**: 182
 3. **Krishnamoorthy, P.**, P.P. Sengupta, M.R. Gajendragad and K. Prabhudas (2011). Pathology of experimental *Trypanosoma evansi* (Leopard isolate) infection in Wistar albino rat. In: South zone conference of Indian Association of Veterinary Pathologists, *National Seminar on “Wildlife pathology” and Workshop on “Diagnostic oncology”*. 30 April – 1st May, 2011, College of Veterinary and Animal Sciences, Pookut, Kerala, India. **pp**: 19.

8232 Popular Articles:

8233 Reports:

8234 Seminars, Conferences and Workshops (Relevant to the Project) in which the Scientists have participated:

(List abstracts forwarded)

1. International symposium on “*Quality assurance in pathology and disease diagnosis*”, 10-12 November 2008, Indian Veterinary Research Institute, Izatnagar, Bareilly, India.
2. Second South Zone Conference of Indian Association of Veterinary Pathologists and National symposium on “**Pathobiology of poultry and laboratory animals diseases**” organized by Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal on 5th and 6th March 2010 at Veterinary College and Research Institute, Namakkal, India.

824 Infrastructural Facilities Developed:

(Details of field, laboratory, notebooks and final material and their location)

Established the facility of animal experimentation and histopathology in the institute

825 Comments/Suggestions of Project Leader regarding possible future line of work that may be taken up arising out of this Project:

Further studies are required with more isolates and animals for the evaluation of pathogenicity of *Trypanosoma evansi*.

Part - IV : Project Expenditure
(Summary)
Year: 2008-2010

830 Total Recurring Expenditure :

8301	Salaries : (Designation with pay scale)		
		<u>Estimated</u>	<u>Actual</u>
	i) Scientific		
	ii) Technical		
	iii) Supporting		
	iv) Wages		
	Sub-total		
8302	Consumables :		
	i) Chemicals	400000	400000
	ii) Glasswares	200000	200000
	iii) Biochemical kits	300000	300000
	Sub-total	900000	900000
8303	Travel:		
8304	Miscellaneous:	200000	200000
	(Other costs)		
	Animal maintenance	100000	100000
8305	Sub-total	300000	300000
	(Recurring)		
831	Non-recurring Expenditure :		
	(Equipment and works)		
	i)		
	ii)		
	iii)		
832	Total	1200000	1200000
	(830 and 831)		

Part - V: DECLARATION

This is to certify that the final report of the Project has been submitted in full consultation with the Project Workers as per the approved objectives and technical programme and the relevant records, notebooks and materials are available for the same.

Signature

of the Principal Investigator: Dr. P. Krishnamoorthy -
Scientist, PD-ADMAS

Co-investigators: 1. Dr. P.P. Sengupta -
Senior Scientist, PD_ADMAS

2. Dr. M.R. Gajendragad -
Principal Scientist, PD_ADMAS

3. Dr. K. Prabhudas -
Project Director, PD_ADMAS

Signature & Comments of the Director:

**Buffalo
Isolate**

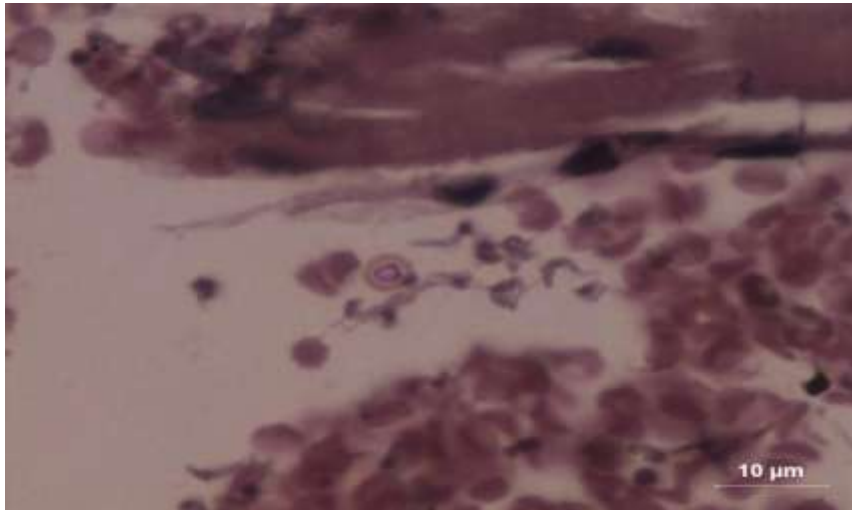


Fig. 1 Heart - *Trypanosoma evansi* (buffalo) in heart chamber musculature (H&E,Scale =10μm)

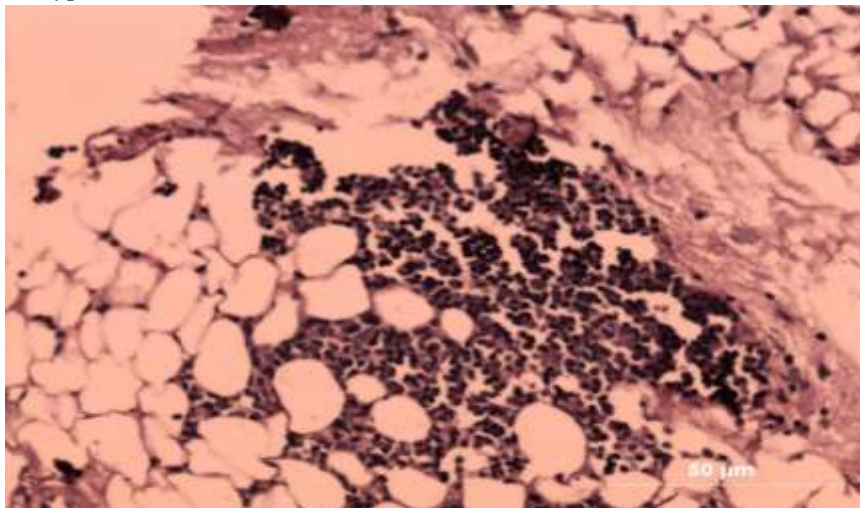


Fig. 2 Lung showing focal mononuclear cell infiltration (H&E,Scale =50μm)

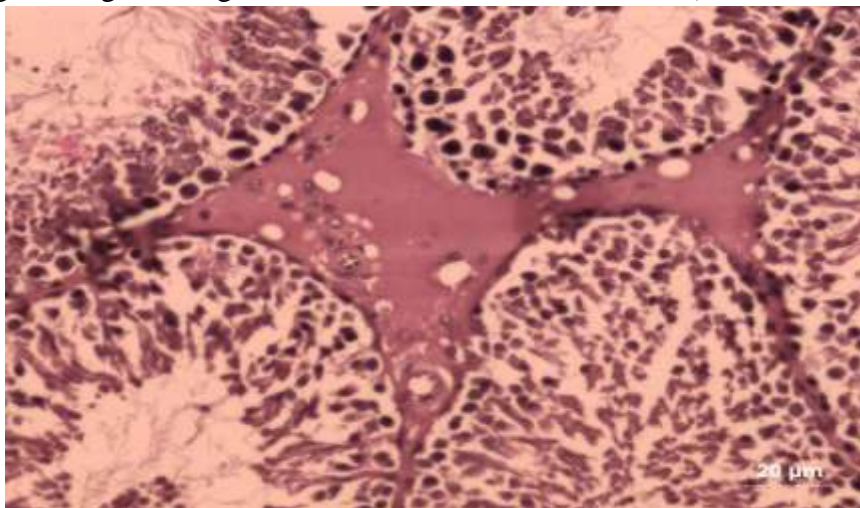


Fig.3 Testis showing edema in the interstitial space (H&E,Scale=20μm)

**Canine
Isolate**

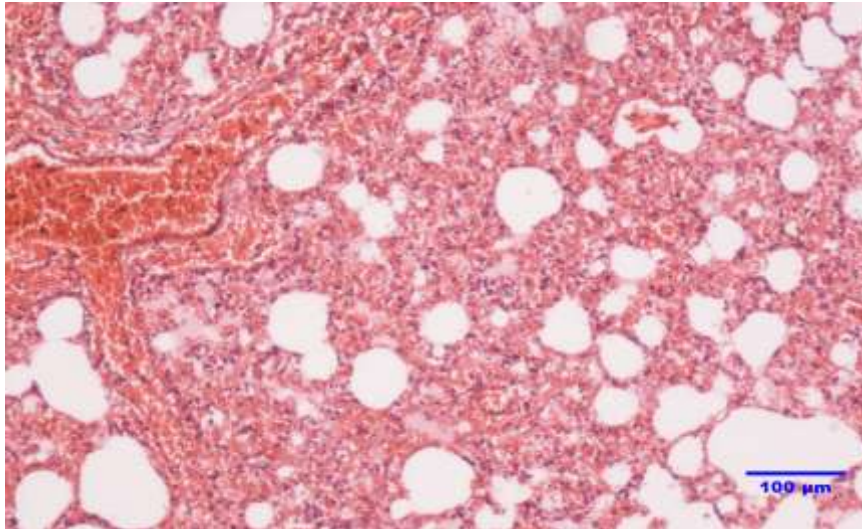


Fig. 4 Rat 5 DPI Lung, alveoli damage and infiltration of inflammatory cells (H&E, scale-100 μ m)

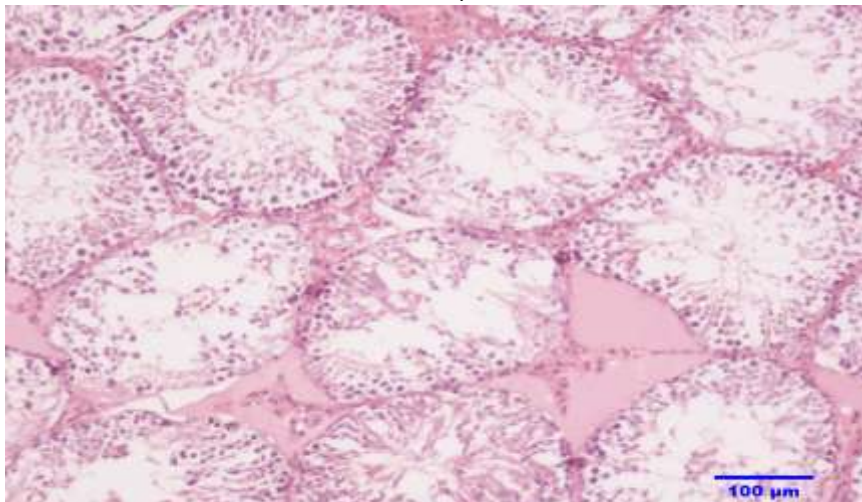


Fig. 5 Rat 6 DPI Testis, interstitial edema and seminiferous tubule degeneration (H&E, scale-100 μ m)

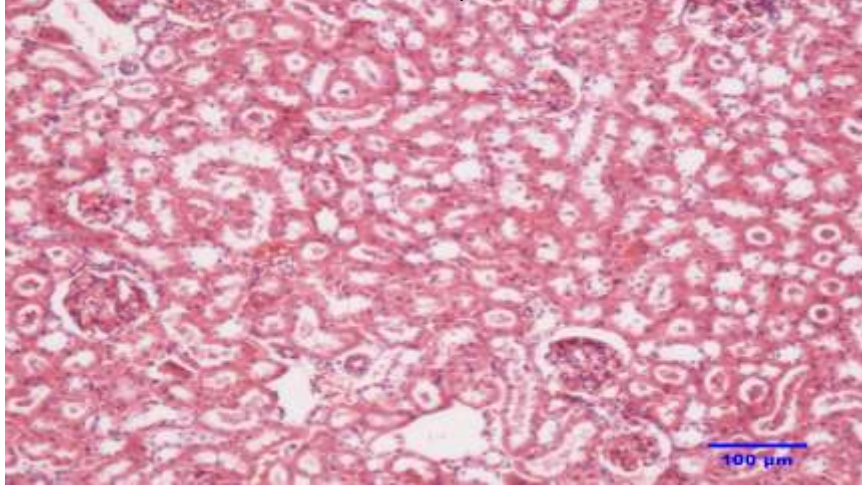


Fig. 6 Rat 7 DPI Kidney, glomerular damage and infiltration of mononuclear cells (H&E, scale-100 μ m)

**Lion
Isolate**

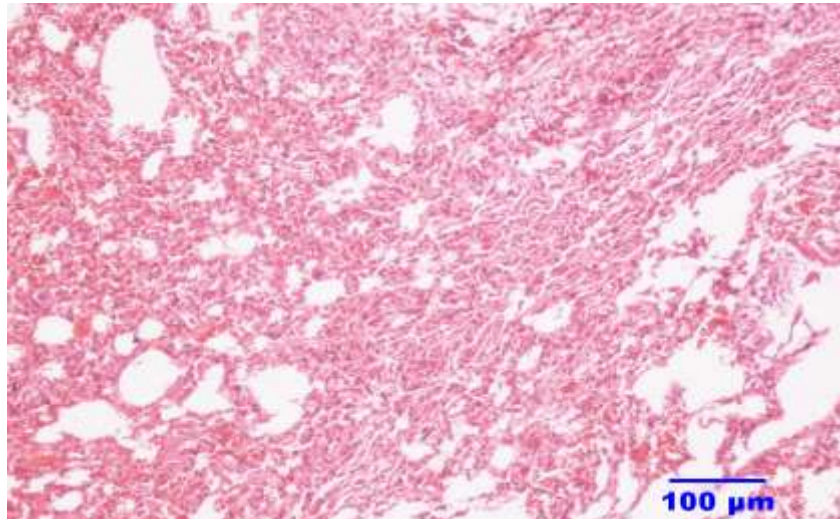


Fig. 7 Rat 7DPI Lung, alveolar damage and inflammatory cell infiltration (H&E,Scale=100 μ m)

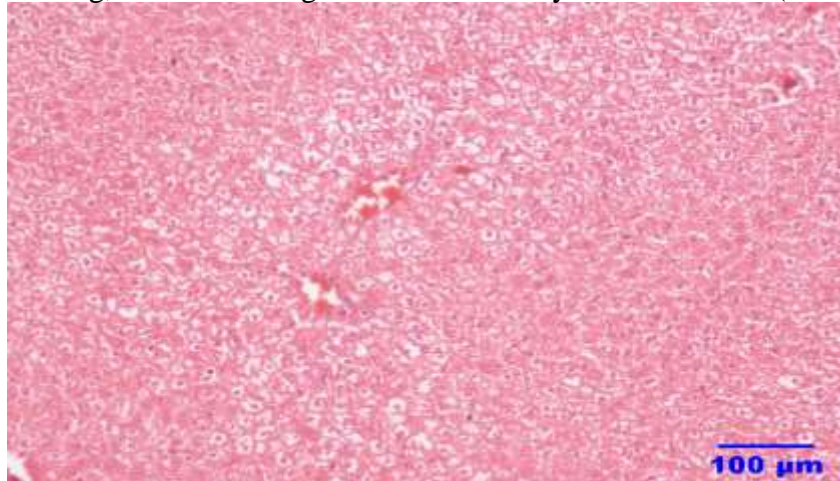


Fig. 8 Rat 7 DPI Liver, severe vacuolar degeneration (H&E,Scale =100 μ m)

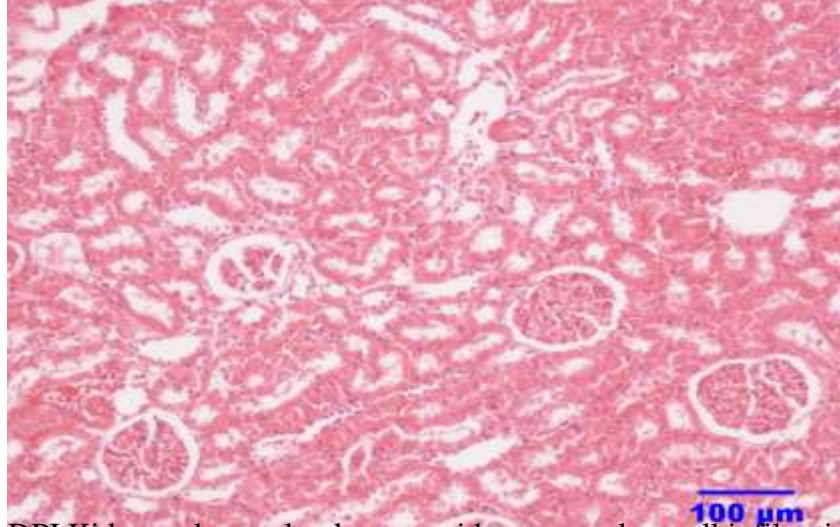


Fig. 9 Rat 7 DPI Kidney, glomerular damage with mononuclear cell infiltration (H&E,Scale =100 μ m)