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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 1 (3): 154-158, 1998 Research Article

Echinococcus granulosus: A Morphometric and Histopathological Response of Kidney in Rabbits

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Abstract

Out of forty rabbits (*Lepus nigricollis*) acclimatized to the optimal condition of animal house, thirty-five were daily inoculated with weekly increasing doses of filtered hydatid cyst fluid of sheep origin for seven weeks. 15 control rabbits were given similar doses of distilled water. Five experimental and three control rabbits were weekly slaughtered and their kidney tissue was fixed in bouins and stained with haematoxylin and eosine. The morphometric findings made and compared with their respective controls, showed significant increase ($p<0.001$) in the area and volume of glomerules. In the histological preparations of kidney of infected rabbits capillaries of the glomeruli, loops of Henle and lumen of collecting tubules was found dilated. The space between the Bowman's capsule diminished while'in some it increased due to the shrinkage of the glomerular capillaries. In the proximal and distal tubules the cell membranes towards the lumen were destroyed while those away from it were diffused. The cells were hypertrophied with colloidal disintegration, vacuolation and in some cases margination. The nuclei were either hypertrophied with vacuolation or pyknotic and darkly stained. The arteries were thick walled and surrounded by fibrotic tissues while the veins were full of red blood cells. Hemorrhage was present in different regions. There was an overall infiltration of leukocytes. It was noted that the damage in the kidney tissues increased with the increase in dose and time.

Key words: *Echinococcus granulosus*, rabbits, kidney, morphometric studies histopathology

Introduction

Hydatidosis, canine associated zoonoses (Cook, 1991) is an important public health threat of global value (FAO, 1985; Thompson, 1986; Andreson *et al*., 1993; Smyth, 1994; Dempster *et al*., 1995; Parada *et al*., 1995; Robert and Janovy, 1996). It is also associated with heavy economic losses each year (FAO, 1985; Schwabe, 1986; Thompson, 1986; Iqbal *et al*., 1989; Andreson *et al*., 1993). Although it is not equally prevalent in all areas (Chi *et al*., 1989) but when the causative agent is once introduced to a region. then it has potential to spread into any geographic area were sheep and cattle raising is carried out with the help of dogs or foxes (FAO, 1985). In Pakistan, besides its seriousness in man (Chaudhry *et al*., 1992; Naveed *et al*., 1993; Junejo *et al*., 1995) and live stock (Khan and Haseeb, 1984; Pal and Jamil, 1986; Iqbal *et al*., 1989) it also results in heavy economic losses each year (Iqbal *et al*., 1989). It lowered the productivity of infected animals and the quality of products obtained from them. Considering their medical, veterinary and economic values an attempt is made to determine some of the morphometric and histopathological changes in rabbits as a model.

Materials and Methods

Maintenance of Rabbits: Fifty-nine healthy rabbit were acclimatized to the optimal conditions of animal house. They were fed on seasonal green fodder twice a day and vegetable kitchen waste once daily along with tap water *ad libitum* to which few crystals of $KMNO_4$ were added to minimize the chances of disease transmission among the animals. A salt brick was provided to supplement their mineral requirements.

Collection of Hydatid Cyst Fluid (HCF): HCF aspirated from the cyst located in lungs and liver of sheep was kept at 4°C to avoid the denatuiation of its enzymes and also to keep the protoscoleces alive. Protoscoleces free or filtered hydatid cyst fluid (FHCF) was obtained by filtering it thrciugh Whatmann filter paper 1 and placed in the deep freezer for further use. Although fresh HCF was collected daily and used for inoculation while freezer stored FHCF was used when the slaughter house remained closed for 2 days in a week.

Dose Inoculation: Increasing doses of FHCF (0.50 ml, 0.75 ml, 1.0 ml, 1.25 ml, 1.5 ml, 1.5 ml, 1.5 ml) were inoculated in the marginal ear vein of 35 experimental rabbits. Each dose was daily inoculated upto one week and then increased while the last three doses were, kept constant. 15 control rabbits were inoculated with similar doses of distilled water.

Three control and five experimental rabbits were slaughtered after the completion of each dose. The 7-8 mm of pieces of kidney were fixed in bouins and Processed for wax embedding, 4-5 micron thick sections were cut and stained with haemotoxyline and eosine (Mahoney, 1973). These sections were scanned under high powered

microscope and morphometric measurements of infected rabbits were noted and compared with their controls by using ocular micrometer at magnification of 400x. Ten measurements at random were taken of the same section and a total of 5 sections were used for each measurements. Glornerulus area (μ)2 and volume (μ)3 was calculated by using formulae nr2 and 4/3nr3. Microphotographs were 'made at an enlargement. 400x. Statistical significance was made by Student 't' test (Steel and Torrie, 1981).

Results

Some mrphometric changes induced by FHCF in the kidney if rabbits were studied. It was noted that area (μ2) and volume (μ3) of the glomeruli showed an increasing trend with lot of fluctuations: Overall increase in the area and volume of glomerulus was 14.96 and 35.82 percent as comiciared to their respective controls (5.14 and 5.41%) respectively ($p < 0.001$) (Fig. 1).

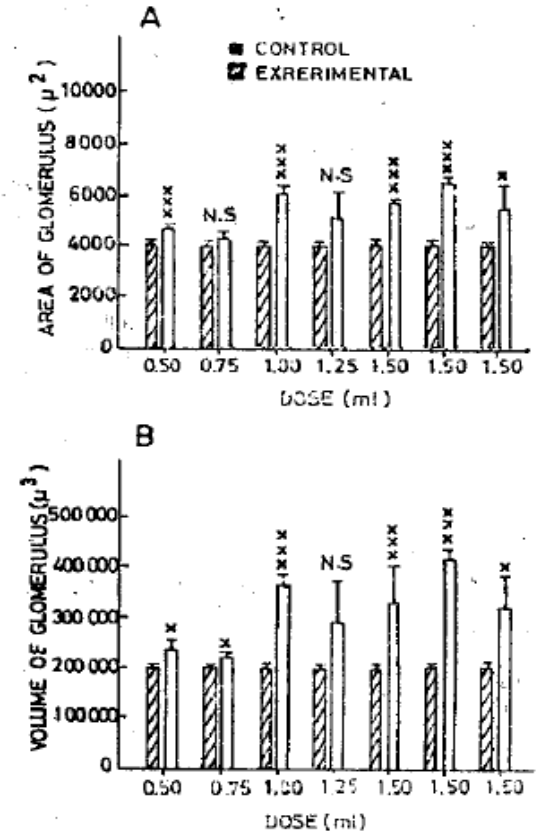

Fig. 1: Morphometric changes in the kidney of rabbits after inoculating different doses of filtered hydatid cyst fluid. The values given are mean ± S.D. of five control and three experimental rabbits. The statistical significance has been determined by the Student 't' test and the probability represented by stars *: $p < 0.05$; **: $p < 0.01$; ***: $F' < 0.001$

The histological preparations of control kidney showed two wgions; the cortex and the medulla. The cortex has the capillaries of the glomeruli enclosed by the Bowman's Capsule with a space 'between them (Plate 1, Fig.1). There were present proximal and distal convoluted tubules in the region along with arteries and veins. The tubules, which touched the glomerulus called the macula dense was occasionally seen. In the medulla, collecting ducts an loops of Henle were present along with arteries and vein: One week after the inoculation of first dose of FHCF slight glomerular nephritis was started with polymorphonuclea leukocytes and monocytes in them (Plate1, Fig.2). With the continuous inoculation of FHCF the loop of Henle were found diluted with mild infiltration of monocytes. The cells were swollen and showed colloidal disintegration an in some cases vacuolation of cytoplasm. Each nucleus possess one to two nucleoli. The endothelial cells wer much enlarged and showed the presence of large vacuoles Cellular necrosis has just started in them. With the passage of time and increasing doses of FHCF, the infiltration o monocytes and leukocytes became intensive. The space between the glomerulus and Bowman's capsule was much reduced. The tubular nephritis was more intense. Some o the capillaries were ruptured thereby releasing blood in the surrounding tissues.

With the increasing doses of FHCF, cortex showed dilate capillaries of the glomerulus and some of them wen ruptured with blood cells in them (haemorrhage) (Plate 1, Fig. 3-9). This condition was only visible in some cases Extensive infiltration of neutrophils and monocytes was not only found in glomerulus but also in tubules. There were some patches in the tissues where heavy infiltration of phagocytes was noted. Both the proximal and distal convoluted tubules showed destruction in the cell membranes directed towards the lumen. The cell membrane of many cells were not distinctly visible. Free nuclei as well as neutrophils and monocytes were occasionally found in them. A large number of macrophage were present in the interstitial spaces. The cells were extensively swollen to such a level that they have almost completely filled the lumen of the tubules. The cytoplasm showed extensive colloidal disintegration and cytoplasmic vacuolation. The nuclei also became extensively hypertrophic. Most of them were pyknotic, deshaped and darkly stained with haematoxylin. The kidney tissue on the whole was necrotic. The arterial walls were thick and were covered with fibrotic tissues with extensive infiltration of mononuclear leukocytes.

Extensive damage and acute necrosis in the medulla region was noted with patches of fibrosis scattered in it. Most of this region had a very heavy infiltration of polymorphonuclear leukocytes and monocytes along with free blood patches. The cells of the loops of Henle and the collecting ducts did not have distinct cell walls and there was prominent colloidal disintegration in the cytoplasm. The lumen of the ducts in most cases were filled with nuclei, polymorphonuclear leukocytes and neutrophils. The nuclear membranes were mostly distinct and the nuclei were pyknotic. The arterial wall also showed fibrosis and the cell membranes were not visible. The veins were full of blood cells (Plate, Fig.7-9).

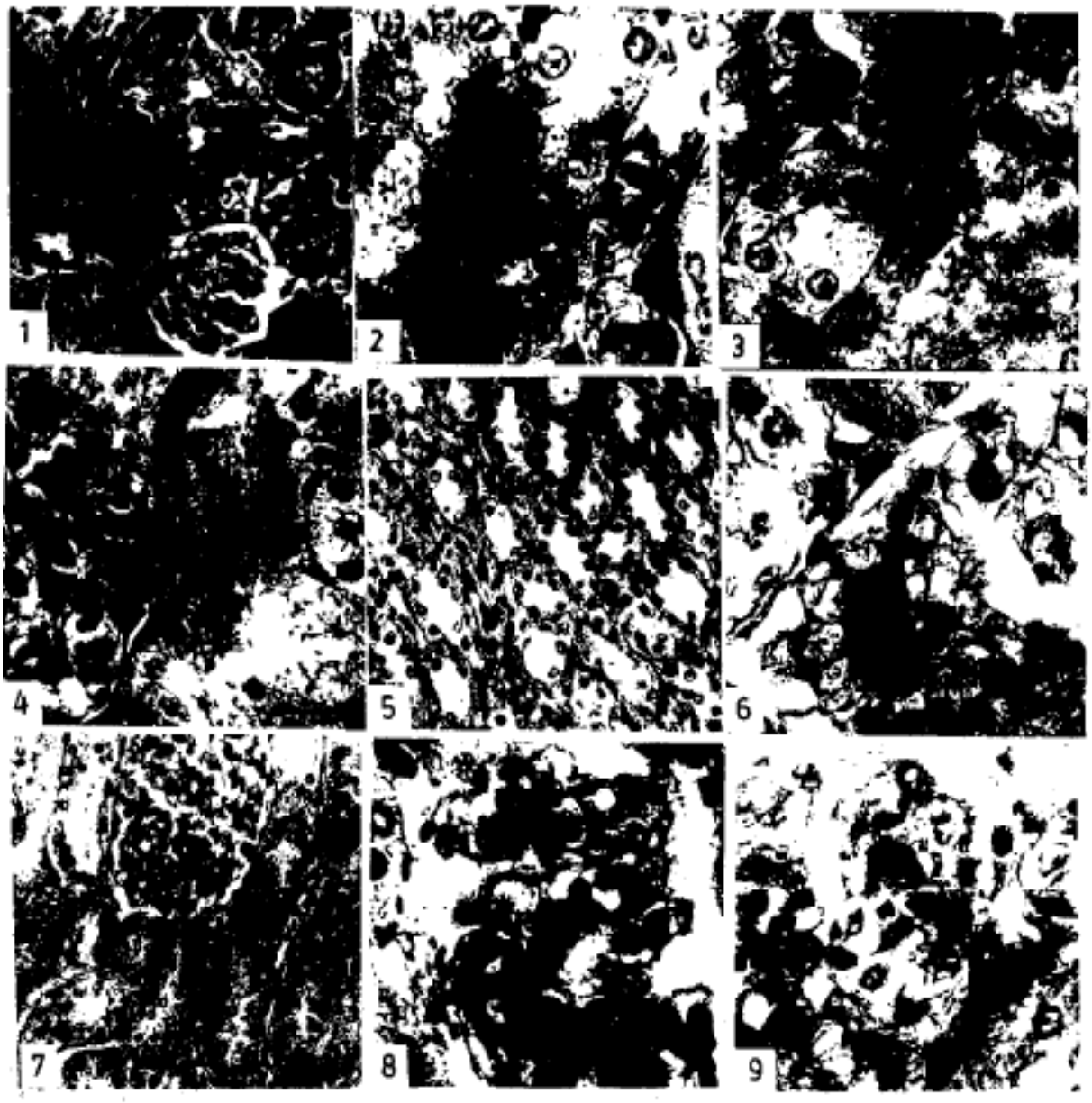

PLATE 1

Plate 1: Showing (1) prominent capillaries of glomeruli and cells and nuclei of convoluted tubultes in the cortex {control rabbits (x 400). (2) sharp and conspicuous outer and diffuse inner membranes of tubules, colloidal disintegration of cytoplasm and some hypertrophied vacuolated nuclei (1) week after inoculation of 0.50 mL FHCF) (x1000). (3) colloidal disintegration and vacuolation of cytoplasm and nucleoplasm in the cortex (2 weeks after inoculation of 0.75 mL FHCF) (x1000). (4) dialated capillaries of a glomerulus infiltered with leukocytes; thick walled cell membrane away from lumen with macrophages between them and deshaped, pyknotic nuclei in the cortex (3 weeks after inoculation of 1.0 mL FHCF) (x1000). (5) dialated loops of Henle and collecting ducts in filtered by leukocytes in the medulla (5 weeks after inoculation of 1.25 mL FHCF) (x400). (6) dilated capillaries of glomerulus packed with leukocytes in the cortex (4 weeks after inoculation of 1.25 mL FHCF) (x1000). (7) colloidal disintegration of cytoplasm, hypertrophic nuclei and broken cell membranes towards lumen of tubules with free nuclei in them, in the cortex (dose, 1.5 mL FHCF) (x400). (8) dialated capillaries of a glomerulus with moderate free space infiltered by leukocytes and hypertrophic cells of tubules closing up the lumen in the cortex (6 weeks after inoculation of 1.5 mL FHCF) (x1000).(9) dialated capillaries of a glomerulus with heavy infiltration of leukocytes; hypertrophic and pyknotic, deshaped and darkly stained nuclei in the cortex 7 weeks after inoculation of 1.5 mL FHCF (x1000)

Discussion

The mammalian kidney is an extremely complex organ, both anatomically and functionally. The primary renal function is excretion of wastes and it plays a significant role in the regulation of total body homeostasis (Klaassen *et al*., 1986). It is internally/ anatomically divided into two areas, the cortex and the medulla. The cortex constitutes a major portion of the kidney and so receives most of the total nutrient blood flow to the organ. Thus, when a blood born toxicant is delivered to the kidney, a high percentage of the material will reach glomeruli in the cortex. The functional anatomy is based on the nephron which consists of the vascular element including the afferent and efferent arterioles, the glomerulus and the tubular element. All nephrons have their primary vascular elements and glomeruli in the cortex. The glomerulus is a specialized capillary bed which is positioned between vasoactive arterioles. It is a relatively porous capillary and acts as a selective filter of the plasma (Klaassen *et al*., 1986). The cytotoxicity of hydatid cyst fluid has been reported in laboratory reared mice, rats and hamsters (Osuna *et al*., 1987) and in the spleen cells of rats (Annen *et al*., 1981). Functionally, toxicity may cause minor alterations in the transport capability. Since the renal blood flow is quite high and both the kidneys receive about 25 percent of the cardiac output. For the maintenance of its normal function, the delivery of large amounts of metabolic substrates and oxygen are required. As salt and water are reabsorbed from the glomerular filterate, the remaining materials including the potential toxicant becomes concentrated (Klaassen *et al*., 1986).

The glomerulus is also susceptible to immunologic injury. The glomerulus acts both as a size-selective and a charge-selective filtration barrier. Following toxic insult changes in glomerular permeability may occur. However, by light microscopy the tissue does not appear to be more porous but somewhat thicker after a toxic insult. In addition, maintenance of normal glomerular ultrastructure is dependent on anion-anion charge repulsion. Reduction of fixed anionic charges allows the terminal divisions of the glomerular podocytes to fuse and accounts for the characteristic thickening of the glomerulus (Brenner *et al*., 1981).

The results of the present investigation showed that after inoculation of high doses of hydatid cyst fluid the area and volume of the glomerulus abnormally increased with the increase in dose and time. Similar findings have also been reported by Brenner *et al*. (1981).

As far as histopathological changes are concerned it was noted that due to the toxins and enzymes present in HFCF there was dilation of the capillaries of glomeruli and the infiltration of leukocytes whose combined effect provide powerful defenses against parasitic infecticIns (Ganong, 1995). The cells of the distal, proximal convoluted tubules, collecting ducts and loops of Henle showed cytoplasmic disintegration, vacuolation and hypertrophy with diffused membranes. The nuclei either became hypertrophic or pyknotic. All these changes may be due to proteinuria as reported by Ozeretskovskaya *et al*. (1978), Vialtel *et al*. (1981) and Ibarrola *et al*. (1981). The enzymes and toxins present in HFCF crossed the glomerular membrane and affected the cells lining all the tubules, collecting ducts and the loops of Henle thereby causing necrosis in them. However, Alkarmi and Ali-Khan (1984) disagreed with such findings and reported that necrosis was never observed in any part of the kidney at any stage of infection (chronic alveolar hydatidosis) in mice. Fibrosis with infiltration of leukocytes was noted in the arteries which had become thick walled. This was also noted by Ali-Khan and Rausch (1987). Hemorrhage was noted in different parts of the kidney tissues probably due to the reduced synthesis of clotting factors by the liver (Walter and Israel, 1987).

Acknowledgments

Financial aid given by Research Committee, Punjab University for the year 1993-1994 is gratefully acknowledged.

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