Current Research Journal of Biological Sciences 1(2): 27-33, 2009
ISSN: 2041-0778

Submit Date: 2009 June, 27 Accepted Date: 2009 July, 05 Publish Date: 2009 August, 24

Immune Mediated and Drug Toxicity Caused Structural Changes in Different Regions of Adjuvant-induced Arthritic Rats Rattus Norvegicus – A Histopathological Study

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Abstract: Rheumatoid arthritis the inflammatory disease of joints and other mucsuloskeletal regions. The exact etiology not fully understood. But, the histopathological examinations giving much anticipated explanation for immune mediated damage. But perhaps, the damage is not only restricted to the joint region. Evidences to structural changes occurred in other regions also well documented. Many pathological changes with other organs such as brain, liver, kidney, spleen and muscle etc., are thought to have similar immune mediated alterations. The Non-Steroidal Anti-inflammatory Drugs (NSAIDs) adverse manifestation is another subject on cause for structural damage for many decades. Till date many works emphasized the need of understanding what could happen in other peripheral and reproductive regions when the inflammation was initiated and maintained in diseased state. Perhaps the inflammation associated changes could also be expected with other regions in the patients and also the NSAIDs are more potent drugs to create undesirable effects when taken regularly. On the one needs to understand the possible pathological changes in connection to inflammation and drug induced changes with disease course. In this present study organs like liver, kidney, testis, muscle, spleen, footpad skin, dorsum skin, knee joint and femur bone were taken for analysis to document pathological changes in adjuvant arthritis condition. The results were documented similar pathological changes in those regions to arthritic knee joint.

Key words: Adjuvant Arthritis, Immune Mechanism, Drug Toxicicity; Inflammation, Histopathology.

INTRODUCTION

The disease rheumatoid arthritis characterized by multiple tissues involvement including brain, liver, kidney, reproductive organs, cardiovascular systems and etc., Histopathological examinations on rheumatoid arthritis is instrumental in understanding immune mediated damage in arthritic foci. But perhaps the damage is not only restricted to the joint region. Convincible evidences to occurance of pathological changes in other regions also well documented. Many pathological changes on organs like brain, liver, kidney, spleen and muscle etc., are thought to have similar to knee joint region.Many histopathological studies in both human subjects as well as in animal models are well documented (Muta and Yamano, 2004; Subramanian and Ramalingam 2002).

MATERIALS AND METHODS

Male Wistar rats weighing 180-220 g were purchased from Animal house, King Institute, Chennai. The institutional ethical guideline is followed. Animals without any symptoms of wound/lesion in the skin or legs were selected for the study. They were housed one per cage with 12 hours light:12 hours dark normal photo period regime in the laboratory. Pellet feed was provided *ad libitum* to the rats. The heat killed Mycobacterium tuberculosis suspension was prepared in the concentration of 2 mg/ml. The bacilli were supplied by the Division of Microbiology, Tuberculosis Research Centre (ICMR), Chennai. The Freund's incomplete adjuvant (FIA) was supplied by the Department of Antitoxin, King Institute, Chennai. To make the Freund's Complete Adjuvant (FCA) 10 mg of heat killed Mycobacterium tuberculosis was mixed with 5 ml of FIA to get 5 ml of FCA. Four groups of six animals, group one representing the normal, group two representing the complete adjuvant treated (FCA), group three represented both complete and incomplete adjuvant treated (FCA+FIA) and fourth group is represented as diclofenac sodium treated (DS). In this, complete and incomplete adjuvant treated group (FCA+FIA) diclofenac sodium treatment given, were taken for the study. The normal/sham treated animals were injected with 0.2 ml of double distilled water at the footpad of right leg on day '0' and kept for 21 days. To the FCA rats injected with 0.2 ml of FCA on day '0'. The FCA+FIA rats given 0.2ml FCA on day zero and same amount of FIA given on day seven at the same right leg foot pad. In the DS rats diclofenac sodium 10µl / day (50µl/5 days) /intramuscularly was given intra-muscularly on days '0', '5', '10' and '15'. Both control and test rats were kept for 21 days and sacrificed on day twenty two by chloroform inhalation. The tissues like liver, kidney, testis, spleen, femur bone, knee joint region, foot pad and

dorsum skin samples were also taken for this study. The tissues were dissected and fixed in 5% neutral buffered formalin solution for a period of 24 hrs. After fixation the tissues were washed over night in running water to remove the fixative. To remove the water content present in the tissues, dehydration was carried out by transferring them to a serious of gradually increasing percentages of alcohol in water. The bone samples and knee joint region were decalcified by nitric acid method and fixed in 70% alcohol. The materials were then cleared in xylol and embedded in wax (melting point 52ºC). The sections were cut at 5µ thicknesses and stained in haematoxylin and eosin coined by Culling (1949).

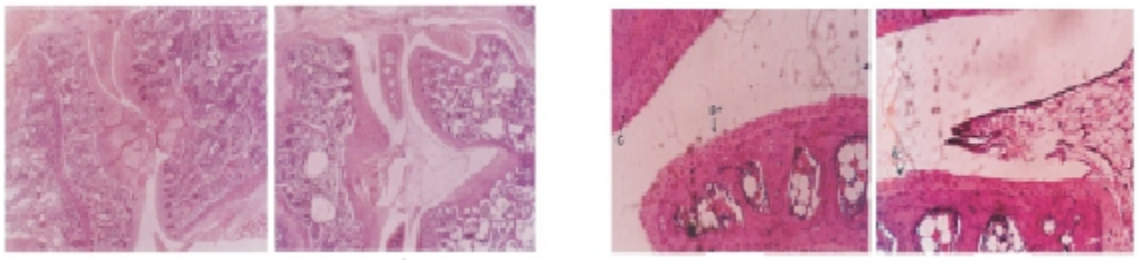
RESULTS

The control Knee joint region showed normal features of the joint cavity. In adjuvant treatment the joint cavity showed pannus formation and damage in the cartilage region along with infilteration of inflammatory cells (Fig.1). Control rats liver showed normal distribution of hepatocytes. But in FCA and FCA+FIA rats amyloid inclusion and kupper cells can be seen around sinusoids and the cells distribution was also found irregular with notable degenerative changes (Fig. 2). In kidney no significant changes noted (Fig 3). The testis showed normal cell arrangement with control rats. But in both FCA and FCA+FIA, the cells showed irregular arrangement, shape and reduced in size (Fig. 4). The control muscle histopathology showed noraml straiated muscle arrangement. But in FCA, FCA+FIA and DS rats, between the striated muscle gaps were found increased and lesion also noticed with FCA treatment (Fig. 5). The control spleen showed normal cell synthesis activity but in adjuvant treatment it shows active cell sysnthesis with no particular structural damage (Fig. 6). The footpad skin of control rats showed with normal features but the adjuvant treated rats showed infiltration in the dermis area and the vacuole formation within the infiltration. While in DS rats the inflamed area showed lesion (Fig. 7). In dorsum skin both inflammation and infiltrations of immune inflammatory cells was noted to adjuvant treatment and vacuolation in the diclofenac sodium treated rats were also seen (Fig. 8). The bone samples were found active cell synthesis (Fig. 9).

DISCUSSION

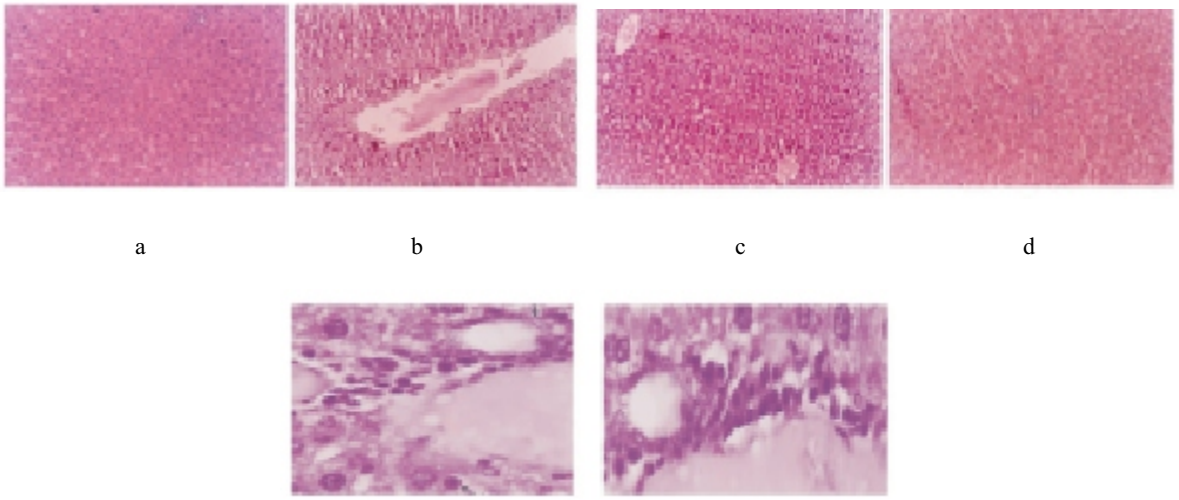
The arthritic manifestation is a peculiar phenomenon without adequate knowledge on its etiology. The pathological changes were well documented by histopathological examinations. The knee joint region charecterized with cartilage damage, pannus formation and infilteration of infammatory cells but it is not restricted to arthritic foci. Many studies have documented pathological changes in other organs also. The liver disturbance is common in collagen diseases. Studies have pointed out that, liver disturbance in rheumatic diseases usually occurs at the on set of the underlying diseases and the severity of liver disturbance can be positively correlated (Kojima *et al*., 2002). Immune activity in other organs may also influence increased inflammatory condition in affected person. Since immune mediated inflammation elsewhere in the body found to be co-ordinated with one another. On the above some experimental evidences needs to be ascertained in order to felicitate a relation between other organs and joints. RA could cause extra-articular manifestations remarkably in the liver region, most importantly, the hepatic disorder is associated with immune mediated hepato-toxicity and hepatic amyloidosis.Arthritis with liver injury generally associated with predominant CD8-positive T cell infiltration (Takahashi *et al*., 2006), lymphocytes (Carpenter and Czaja 2002), antibodies against cyclic citrullinated peptides (Montano *et al*., 2006), leukocytosis and eosinophilia, anti-nuclear antibody, anti-SS-A antibody and anti-SS-B antibody (Yokota *et al*., 2006). Similarly severe liver damage accomponied with elevated IgG to nuclear and smooth muscle antigen (Ohira *et al*., 1998), anti-nuclear antibodies, high titers of anti-Scl-70, anti-SS-A, anti-centromere, anti-mitochondrial M2 antibodies (Kogawa *et al*., 2005), and increased large granular lymphocytes (LGL) CD3(+)CD8(+) (Tabata *et al*., 2006). Infiltrated T lymphocytes with similar levels of both CD4+ and CD8+ cells and plasma cells positive for anti-IgG4 monoclonal antibodies also documented (Fukui *et al*., 2005). In addition significantly higher level of IL-8 (Chen *et al*., 2004), leukocytosis, negative rheumatoid factor and antinuclear antibodies (Leibovitch 2000) were also given for more understanding on liver pahtology and immune mechanism.

In addition, B-cell clonality in the liver attributable to antigen-driven clonal expansion (Tokuno *et al*., 2003). Sialoadhesin, sialic acid binding immunoglobulin (Ig)-like lectins expressed observed in tissue macrophages of spleen, lymph node, bone marrow, liver, colon, and lungs in arthritic condition (Hartnell *et al*., 2001). Furthermore, defective fas-mediated apoptosis caused deleterious effects in liver with severe hepatic injury in mice (Ichikawa *et al*., 2000). Liver biopsies of patients with Autoimmune cholangitis (AIC) characterized with antibodies against many immune components and predominant T cells in the portal inflammatory infiltrate and in the bile duct epithelium with inflammatory bile duct destruction observed (Kaserer *et al*., 1998). Concomitantly Liver biopsy showed Kuppfer cell hyperplasia, portal and sinusoidal infiltrates of mononuclear cells (Gallo *et al*., 1997). In addition patients suffering from nodular regenerative hyperplasia of the liver characterized with small-sized hepatocytic nodules scattered throughout the liver (Rougier *et al*., 1978). In the present study, in liver amyloid inclusion and kupper cell infilteration were seen. This indicates the immune mediated pathological changes of the present study.



a b c d

Fig 1: Control Knee Joint Region Shows normal architecture of the knee region; b,c,d-Adjuvant lnduced Arthritic Knee joint Region Show infilteration of inflammatory cells, pannus formation and cartilage damage. C-Cartilage;E-Erosion .(H&E; 200X Magnification)



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d f

Fig 2: a-control ; b-FCA induced arthritis; c-FCA+FIA induced arthritis; d-Diclofenac Sodium treatment. b-Shows severe damage in sinusoid region and possibly with amyloid aggregate; e&f-shows amloid inclusion around sinusoid in adjuvant treatment. Arrow shows kuffer cell and a lymphocytes (H&E Staining; 200X Magnification

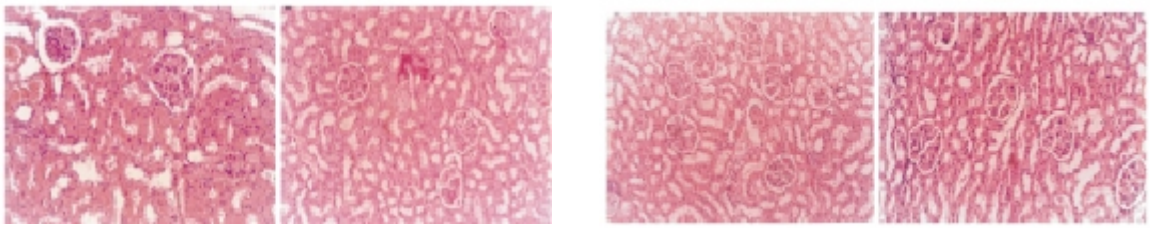

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Fig 3: a-control; b-induced arthritis; c-FCA+FIA induced arthritis; d-Diclofence Sodium treatment (H&E Staining; 200X magnification

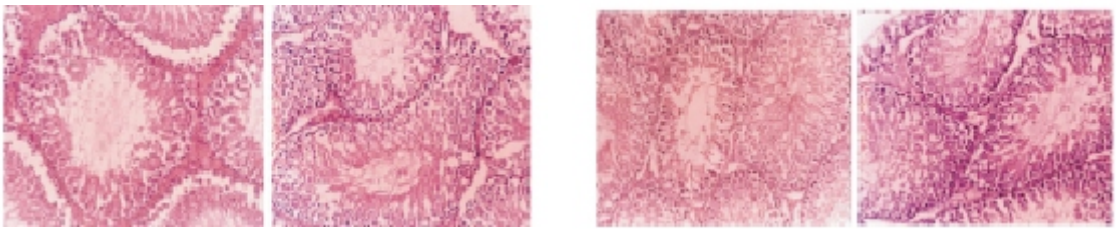

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Fig 4: a-control; b-FCA induced arthritis; c-FCA+FIA induced arthritis; d-Diclofenac Sodium treatment (H&E staining; 200X magnification)

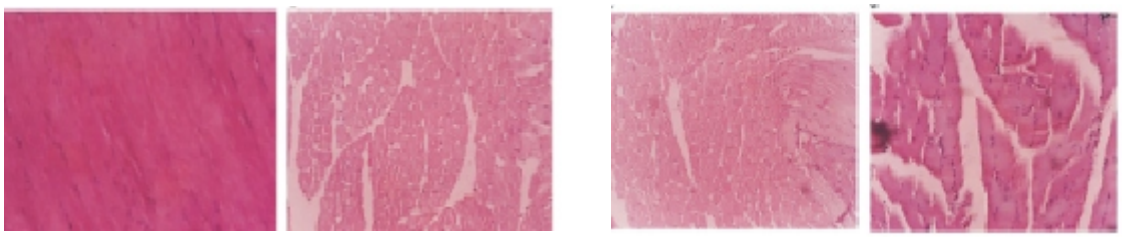

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Fig 5: a-control; b-FCA induced arthritis; c-FCA+FIA induced arthritis; d-Diclofenac Sodium treatment (H&E staining; 200X magnification)

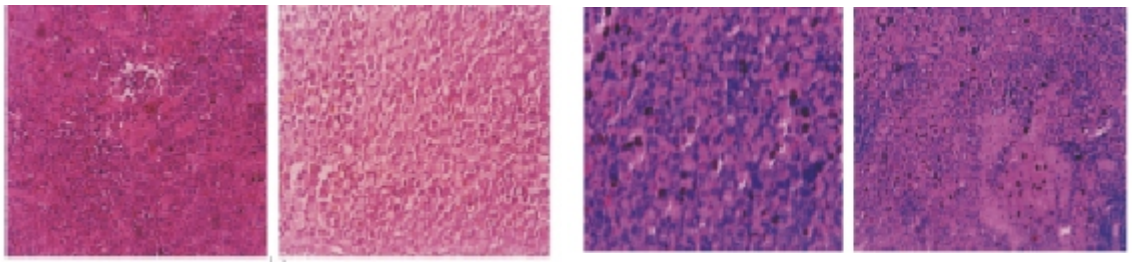

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Fig 6: a-control; b-FCA induced arthritis; c-FCA+FIA induced arthritis; d-Diclofenac Sodium treatment (H&E staining; 200X magnification)

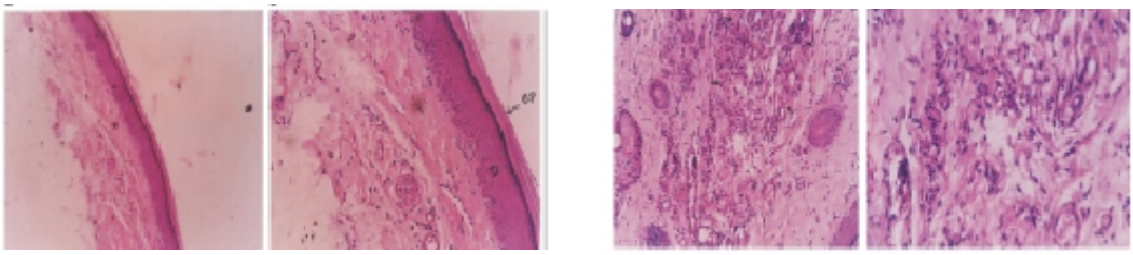

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Fig 7: a-control; b-FCA induced arthritis; c-FCA+FIA induced arthritis; d-Diclofenac Sodium treatment EP-Epidermis; D-Dermis. (H&E staining; 200X magnification) The region with in arrows showed infilteration of flammatory cells like neutrophils and mast cells.

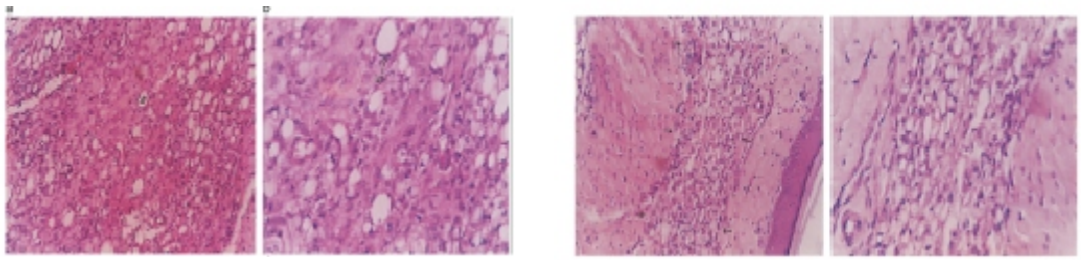

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Fig 8: a-control; b-FCA induced arthritis; c-FCA+FIA induced arthritis; d-Diclofenac Sodium treatment va:vacuoles.(H&E staining; 200X magnification)

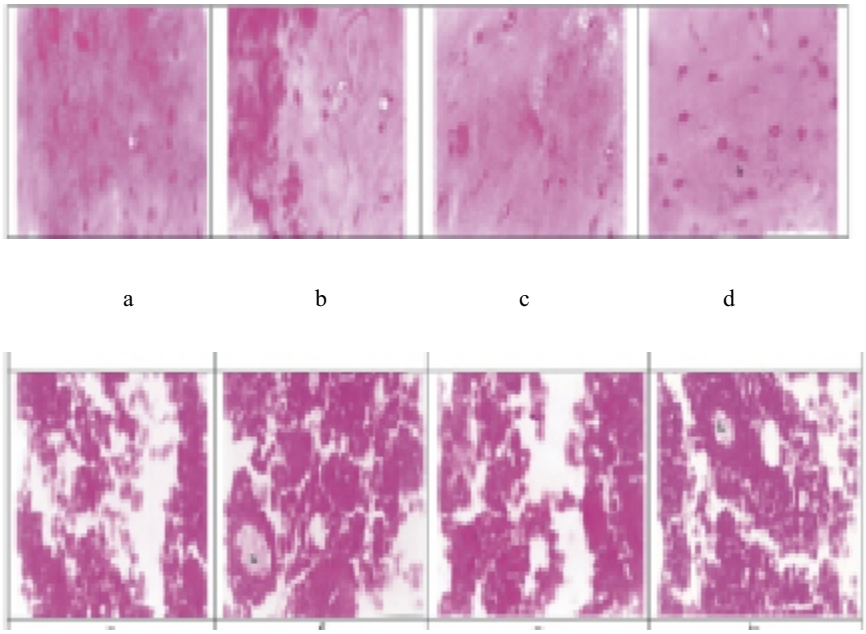

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Fig 9: a & e -Control rat bone and bone marrow histology; b&f -FCA rate bone marrow histology; c&g-FCA+FIA rats bone and bone marrow histology; d&h-DS rats bone and bone marrow hostology. Os-Osteocytes; BI-Blood Vessel

However, the pathological changes may be attributable to drug induction. Lysosomal enzymes association to pathological changes in different tissues like liver, kidney and spleen in adjuvant induced arthritic rats documented (Rao 1995); Subramanian and Ramalingam 2000). The increased activity of aspartate aminotransferase (AST) in peripheral regions could be expected to occur associated with necrosis. AST is significantly increased in such cases and escapes to the plasma from the injured cells (Hassoun and Stohs, 1995; Subramanian and Ramalingam 2003). Therefore the elevated activity of AST and alanine aminotransferase in plasma may be mainly due to the leakage of those enzymes from the liver cytosol into the blood stream. The elevated activities of lactate dehydrogenase (LDH) and acid and alkaline phosphatase (ALP) also (Takahashi *et al*., 2006; Subramanian and Ramalingam 2003) implicate severe damage to peripheral environment.

Similarly peripheral organ functional abnormalities are associated with methotrexate like anti-inflammatory drugs use in rheumatoid arthritis (RA) resulted with clinically significant side effects (Yazici *et al*., 2005). Peirpheral environment damage associated with maximum activity of alanine, aminotransferase and ALP with significant changes in liver histology (Kojima *et al*., 2002). Rheumatoid arthritic patients showed baseline mild perisinusoidal fibrosis to methotrexate (MTX) treatment. In EM studies increased collagen fibers in the disease spaces also documented (Ros *et al*., 2002). Likewise Electron microscopy (EM) analysis of long-term methotrexate (MTX) treatment showed neutral fat, secondary and tertiary lysosomes, and smooth

endoplasmic reticulum (SER) in hepatocytes. Also collagen in the perisinusoidal space showed elevated aspartate transaminase, alkaline phosphatase, bilirubin, and albumin levels in above subjects (Kremer and Kaye 1989). Though the lysosmal origin of proteolytic and hydrolytic enzymes are responsible for the damage in the peripheral environment, the connection between immune mechanism for exitation of the above enzymes activity were not uncommon. Besides immunoglobulin mediated lysosomal enzyme activity includes alkaline phosphatases which thought to be present with immune complex.

In the present study the bone histology of adjuvant treated rats showed no significant changes in structural details compared to that of control,but thebone marrow showed development ofblood vessel. The above angiogenesis is indicative active inflammatory cell synthesis. The knee joint region showed pannus formation with infiltration of inflammatory cells. Erosion of the cartilage surface area near the pannus is also noticed. Chew *et al*., (1990) have identified infiltrated plasma cells, macrophages and polymorphonuclear cells in the developing pannus. The enzymes responsible for the destruction of the articular cartilage may be origin of above cells (Mohr 2003).

CONCLUSION

The pathological changes observed in the present study could be correlated with either immune mediated or drug induced pathological manifestation. Though the immune mechanisms can recruite inflammatory cells to destroy immune complex and vice verse the proteolytic and hydrolytic enzymes of polymorphs origin could damage the residual tissue. Otherwise it may be of drug in duded over activity of the tissue to over come drug toxicity. The drug toxicity always notible with kidney microtubules but in this present study no particular damage was noticied with kidney samples. Eventhough the drug toxicity cannot be taken lightly, hence they can create such damage in some tissues like testis. The present study showed irregular shape in testicles may be associated with drug toxicity. The brain region also showed some pathological changes, which could be attributable to immune mechanism because the inflammation can affect brain while breaking blood-brain barrier. The amyloid inclusion in brain region is common with higher activity of inflammation. The amyloid precursors are nothing but the remnant of the immune complex. Those if not removed activity, they use to bind together and coil. This feature cannot be taken by circulation and vice verse recruits secondary inflammation.

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