

Study of Histopathological & Physiological Effects of Leflunomide on Kidneys in Male Albino Mice

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ABSTRACT

Aim: To observe the effect of exposure of leflunomide on kidney, serum levels of creatinine & urea by giving variable doses of leflunomide.

Methods: Thirty adult male albino mice were divided into three groups. The first group considered as control group was given normal saline orally. The second group was given leflunomide orally (20mg/kg b.w) & leflunomide was given orally (60mg/kg b.w) to the third group. Two physiological parameters were checked i.e., serum urea & creatinine levels.

Results: The results revealed significant increase in serum urea level ($P < 0.05$) in second group compared to control group & significantly raised serum urea level ($P < 0.05$) in comparison to control group. A significant increase ($P < 0.05$) in serum urea level was observed in third group compared to second. No significant ($P > 0.05$) increase was observed in serum creatinine level in second group compared to control where as significantly increased ($P < 0.05$) serum level of creatinine was seen in third group compared to control. Light microscopic study of kidneys of second group revealed enlargement of renal tubules & mononuclear cells infiltration between renal tubules. The third group showed acute cellular degeneration of epithelial lining of renal tubules with glomerular atrophy. Neutrophils are also present in congested blood vessels between tubules.

Conclusion: The results of study confirmed that leflunomide caused a significantly raised serum urea & creatinine levels. Light microscopic changes on kidney tissue of treated mice were also observed.

Keywords: Leflunomide, male albino mice, kidney

INTRODUCTION

Leflunomide is a disease modifying anti-rheumatic drug. Leflunomide is a pyrimidine synthetic inhibitor which is available as tablets containing 20 & 100 mg of active drug. It is a drug which inhibits the enzyme dihydro-orotate dehydrogenase (DHODH) (Hirashiba et al; 2007). It has anti proliferative activity and anti inflammatory effect which slows progression of disease and relief of symptoms of RA. After oral administration it is converted into active metabolite teriflunomide in liver at cytosolic and microsomal sites and excreted through renal and biliary routes (Pinto, P 2006). Other studies regarding Systemic lupus erythmatosis, Felty's syndrome, ankylosing spondilitis, crohn's disease and sarcoidosis have been conducted.

The kidneys are bean shaped organs & consists of nephrons, tubular system & collecting ducts. Nephrons form the basic structural & functional unit of the kidneys. Urea & creatinine are waste products of aminoacid metabolism & removed by the kidneys.

Serum urea & creatinine levels are helpful in diagnosis of kidney diseases. The infection can be diagnosed by identifying changes in histological sections or by performing serological tests. The present study was performed to evaluate both histopathological changes in kidney tissue & changes in kidney function by performing serological tests.

MATERIALS & METHODS

Thirty albino male mice eight weeks old were obtained from animal house of University of Health Sciences Lahore. They were kept in iron cages in well ventilated room, temperature of 28 C under day & night cycle of twelve hours each. They were divided into three groups each containing ten rats. First (control) group was treated with normal saline. Second group was treated with leflunomide (20mg/kg b.w) for thirty days & group three received (60mg/kg b.w) for thirty days.

Histological examination: Rats were sacrificed kidneys were isolated and fixation was done in 10% neutral buffered formalin for 24 hours. Then tissue was dehydrated through ascending & descending series of ethanol, washed & embedded in paraffin. One micrometer thick section was obtained & stained

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with eosin & hematoxylin (H&E) for histological examination.

Collection of blood sample for biochemical tests: Blood sample was taken by a syringe from carotids & serum was separated by centrifugation to evaluate serum urea & creatinine levels. Kits were purchased for urea & creatinine. Urea was determined by

enzymatic & creatinine was determined by kinetic method.

Statistical Analysis: Data was analysed by analysis of variance using SPSS version 20. Variables were expressed as mean±SE. A P value of <0.05 was taken as statistically significant.

RESULTS & DISCUSSION

Serum urea & creatinine level estimation

Parameters	Group 1 (Control) (Mean±SE)	Group 2 (20 mg/kg) (Mean±SE)	Group 3 (60mg/kg) (Mean±SE)
Urea mg/dl	46.4± 0.4	55.7± 0.6	67.7± 0.4
Creatinine mg/ dl	0.54±0.04	0.60±0.02	0.77± 0.02

The results in table revealed significantly raised (P <0.05) in group 2 compared to control group (55.7±0.6mg/dl) & significantly increased urea level in group three (67±0.4mg/dl) was observed as compare to control. Blood urea was also markedly increased in group three as compare to second group. The table also showed marked increase in blood creatinine level in third group (0.77±0.02) as compare to control (0.54±0.04) but there was no significant rise in creatinine level in group two (0.60±0.02) in comparison to control group (0.54±0.04). Serum urea & creatinine was raised due to toxic effects of leflunomide on kidneys.

Histopathological examination: Present study revealed light microscopic changes in kidney tissues. Section of control mice showed flat capsular epithelium. Glomeruli are normal with lobular organization & tubules are lined by cuboidal epithelium (Fig. 1).

The histopathological effects of leflunomide on kidneys of mice treated with 20mg/kg are presented in figure 2& 3 which revealed mononuclear cell infiltration & enlargement of renal tubules. The light microscopic changes in group three were seen in figure 4&5 characterised by vacuolar degeration of epithelial cells, atrophy of glomerulus & congested blood vessels are seen between tubules. The negative effects in tissue sections treated with leflunomide may be due to role of kidney in excretion of metabolites. The transendothelial migration of inflammatory cells to site of inflammation due to leflunomide caused mononuclear cell adhesion.

Fig. 1: Histology of normal kidney (control)

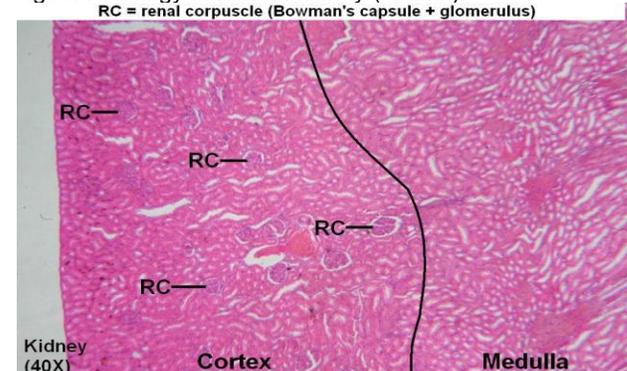


Fig. 2: Lymphocytic infiltration & enlargement of renal corpuscle

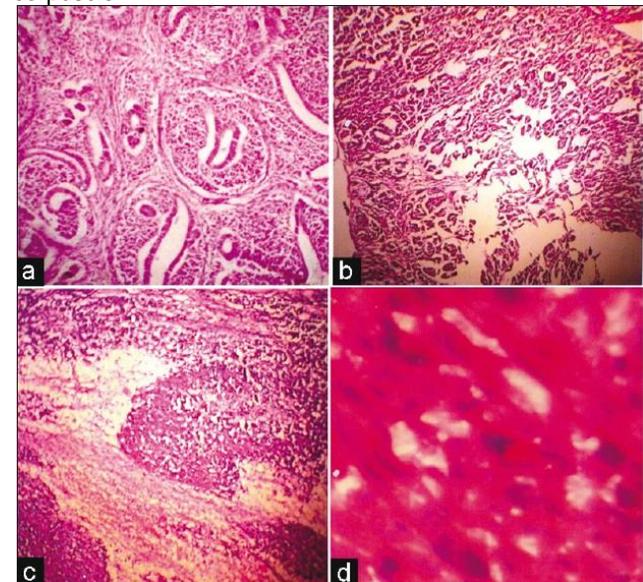
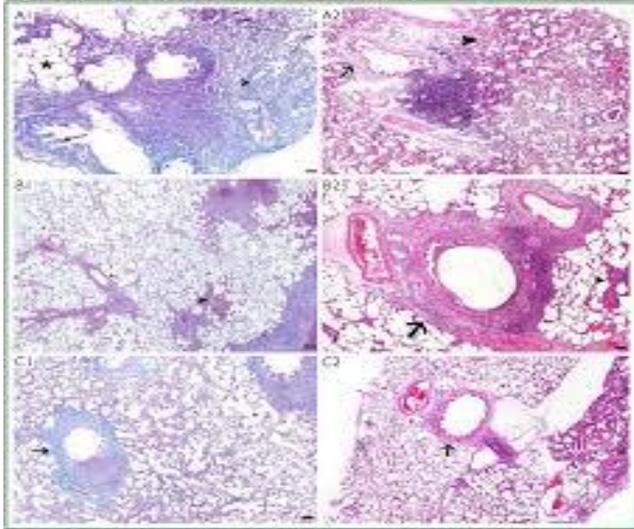


Fig.3: Lymphocytic infiltration & atrophy of renal tubules



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