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**ORIGINAL ARTICLE**

# Mercury Kushta Induced Histopathological Changes in Kidneys of Wister Rats

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## ABSTRACT

**Objectives:** To see the histological changes of Kushta which contains mercury, on kidneys of wister rats

**Material and Methods:** A total of 42 Wistar rats were used in this animal experiment, which was separated into five exposure groups and one control group. The kidneys of rats were morphologically altered by the use of both indigenous and prescription mercury preparations.

**Results:** Morphological changes in kidneys of exposed rats included multi-focal and diffuse glomerulonephritis, mesangial widening, increase in glomerular cellularity, thickening of capillary walls and glomerular basement membrane, tubular necrosis, tubular dilatation, tubular vacuolisation, proteineous casts in tubules and mononuclear interstitial inflammation were seen at the end of 8 weeks.

**Conclusions:** In wister rats, an indigenous mercury herbo-mineral preparation (Kushta) causes harmful morphological changes in the liver.

**Keywords:** Mercury Kushta, Kidneys, Histopathology

## INTRODUCTION

Naturally metallic heavy elements occur in earth crust and also accumulate in human bodies and plants in trace amount[1]. Animals require these elements e.g. selenium, zinc, copper, iron etc. for proper functioning at cellular levels. Even at low concentrations, heavy metals like cadmium, arsenic, mercury, lead, and chromium are poisonous and dangerous to people[2].

In pharmacology, these elements and their compounds have had a long history. Over the past two thousand years, the three main systems of medicine in South East Asia (Unani-Tibb, Ayurveda, and Siddha) have all utilised them. Various illnesses, both common and rare, have been treated with these heavy metals and their derivatives, which are utilised both internally and externally[3].

For many years mercury is being utilized commercially and for pharmaceutical purposes. This substance is found in hospital thermometers and blood pressure cuffs. Batteries, fluorescent lamps, and switches all contain lithium ion batteries. In the electrolytic generation of sodium hydroxide and chlorine from saline, metallic mercury is employed in electrodes. Advances in toxicology have helped us better understand mercury, which was once a frequent ingredient in many medicines used to treat a variety of ailments[1-6].

One of the practices by “Hakeems” the traditional healers is that they prepare a specific form of medicine. It is prepared by heating of different kind of elements and minerals with different plants and herbs and then grinding them vigorously. Kushta in the Unani system and Bhasma in the Ayurvedic system refer to these herbo-mineral medicines. The Persian term "Kushtan," from which this word "Kushta" is derived, means "to slay" or "to conquer." This these types of preparation constitutes of toxic and hazardous on intake by clinical experts are oxidised metals such as zinc (lead), arsenic (tin), and mercury (zinc)[7,8].

Traditional texts state that the detoxification, purification, and restoration of medicinal characteristics are accomplished by physiochemical processing of various kinds of preparations. Kushta preparations commonly just state the element type without detailing the chemical nature, substance, or intermediate product involved[3,7].

Mercury kushta, or "kushta Para," is a popular name for the substance. To treat asthma, impotence, paralysis, and cough, the Hakeems turn to this particular variety of kushta. The kidneys of wister rats are the focus of this study, which examines the histological consequences of kushta containing mercury.

## MATERIAL AND METHODS

In the Department of Pathology, University of Health Sciences, Lahore, Pakistan, a six-month experiment was carried out.

The University of Veterinary and Animal Sciences, Lahore, provided 42 Wistar rats between the ages of six and eight weeks, each weighing between 200 and 250 grammes. Six groups of seven rats each were formed at random. The experimental groups II, III, IV, V, VI, and VII were separated from the Control Group – I. For eight weeks, these groups were given mercury kushta. Table-I provided the details of the feeding schedule. For eight weeks, this was administered on alternate days. At the beginning of the trial in groups IV and VI, BSA (Bovine Serum Albumin) injections were administered and metallic preparations were administered after 2-3 hours. The permeability of capillaries increases when BSA is injected. The recommended dosage is 250mg/kg of body weight each day[8].

Table-I: BSA: Bovine Serum Albumin

| Group | Feed |
|---|---|
| I | Flour diet in pallet forms |
| II | Diet + 0.15 mg Hg kushta on alternate days |
| III | Diet + 0.3 mg Hg kushta on alternate days |
| IV | Diet + BSA i.v once at the start + 0.3 mg Hg kushta/day |
| V | Diet + .0.5 mg of inj. Mercuric chloride 3 times/wk i.p |
| VI | Diet + 250 mg BSA/kg body wt i.v. once at the start + 0.5 mg of inj. Mercuric chloride 3 times/wk i.p. |

Intra-peritoneal IP
Intra-venous IV

Using ether anaesthesia, the kidneys of the rats were removed under aseptic conditions, rinsed with normal saline to remove any blood contaminants, and then placed in formalin for a period of 72 hours. The organ was cut into 3 to 5 mm parts, and each section was labelled and passed in a single tissue cassette. These cassettes were processed in accordance with the normal procedure." Histopathological slides were stained with H & E as well as methenamine and PAS for standard histopathology. The diagnosis was made after microscopic examination of slide specimens. Proformas were used to record findings.

## RESULTS

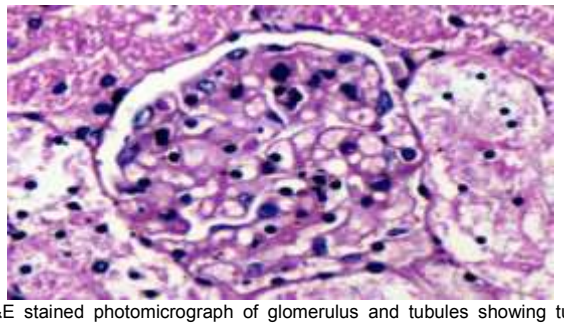
Gross examination revealed no abnormalities. Each kidney segment was examined microscopically for the following histological findings (Table II).

Table-II: Each wistar rat's kidneys were examined for histopathological abnormalities

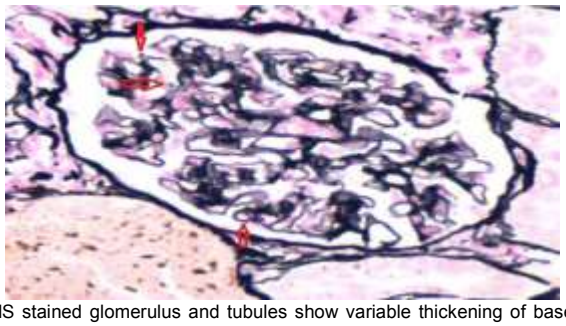
| |
|---|
| Glomerular Cellularity |
| Tubular Necrosis |
| Glomerulonephritis |
| Mesengial widening |
| Thickening of capillary wall |
| Thickening of basement membrane |
| Tubular dilatation |
| Tubular Vacuolisation |
| Protein casts in tubules |
| Interstitial Inflammation |

Data was gathered and organised in a table (Table III). Mercury kushta and injection mercuric chloride have been found to be strongly linked (p = 0.0001) with the anomalies observed after 8 weeks of exposure. i.e. Glomerulonephritis, thickening Glomerular capillary walls and basement membrane, renal tubular dilatation and vacuolization in kidneys of Wistar rats.

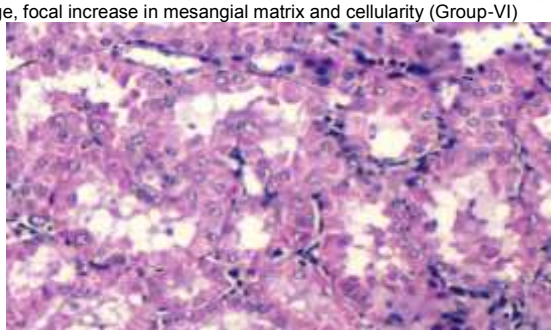
Injectable mercuric chloride and the prescribed dosage of mercury kushta were shown to be substantially linked (p 0.001) after 8 weeks of exposure with mesangial widening and Glomerular cellularity, $p$ = 0.008 with tubular epithelial necrosis, $p$ = 0.007 with protein casts in tubules and $p$ = 0.005 with renal interstitial inflammation. At the end of the trial period, kidney morphological parameters were virtually completely disturbed in all study groups, no matter the dosage of *Mercury kushta*.



**A:** H&E stained photomicrograph of glomerulus and tubules showing tubular damage, focal increase in mesangial matrix and cellularity (Group-VI)



**B:** JMS stained glomerulus and tubules show variable thickening of basement membrane. Arrows showing irregular basement membrane deposits (Group-VI)



**C:** H&E stained micrograph showing tubular Vacuolisation and damage (Group-V)

**D:** JMS stained micrograph showing irregular basement membrane thickening & focal mesangial widening with increase in basement membrane like material (Group- IV)

Table-III: kidney histopathological alterations within each rat strain. (In each column, the number of rats is listed)

| Histopathological Characterstics | | Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | Sub total | Total |
| Glomerular Cellularity | None | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 42 |
| | Mild | 0 | 6 | 2 | 1 | 0 | 0 | 9 | |
| | Moderate | 0 | 1 | 5 | 6 | 4 | 2 | 18 | |
| | Severe | 0 | 0 | 0 | 0 | 3 | 5 | 8 | |
| Tubular Necrosis | None | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 42 |
| | Mild | 0 | 3 | 0 | 0 | 0 | 0 | 3 | |
| | Moderate | 0 | 4 | 5 | 6 | 3 | 0 | 18 | |
| | Severe | 0 | 0 | 2 | 1 | 4 | 7 | 14 | |
| Glomerulonephritis | Absent | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 42 |
| | Multifocal | 0 | 7 | 6 | 7 | 7 | 7 | 34 | |
| | Diffuse | 0 | 0 | 1 | 0 | 0 | 0 | 1 | |
| Mesengial widening | Absent | 7 | 0 | 0 | 0 | 7 | 0 | 14 | 42 |
| | Present | 0 | 7 | 7 | 7 | 7 | 7 | 35 | |
| Thickening of capillary wall | Absent | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 42 |
| | Present | 0 | 7 | 7 | 7 | 7 | 7 | 35 | |
| Thickening of basement membrane | Absent | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 42 |
| | Present | 0 | 7 | 7 | 7 | 7 | 7 | 35 | |
| Tubular dilatation | Absent | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 42 |
| | Present | 0 | 7 | 7 | 7 | 7 | 7 | 35 | |
| Tubular Vacuolisation | Absent | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 42 |
| | Present | 0 | 7 | 7 | 7 | 7 | 7 | 35 | |
| Protein casts in tubules | Absent | 7 | 0 | 0 | 2 | 2 | 1 | 12 | 42 |
| | Present | 0 | 7 | 7 | 5 | 5 | 6 | 30 | |
| Interstitial Inflammation | Absent | 7 | 0 | 4 | 2 | 0 | 0 | 13 | 42 |
| | Present | 0 | 7 | 3 | 5 | 7 | 7 | 29 | |

## DISCUSSION

Toxic effects caused by heavy metals such as mercury are well-known and established in the field of experimental animal physiology. An investigation of kidney morphology in Wister rats exposed to an indigenous herbo-mineral unani preparation of Mercury, known as Mercury kushta, was carried out and compared to the effects of Mercuric Chloride.

In the present study, multi-focal and diffuse glomerulonephritis, mesangial widening, moderate to severe increase in glomerular cellularity, thickening of capillary walls, thickening of glomerular basement membrane, moderate to severe tubular epithelial cell necrosis, tubular dilatation, tubular vacuolisation, proteinaceous casts in renal tubules and various grades of mononuclear inflammatory infiltrate in the interstitium were found in the majority of rats exposed to Mercury kushta for 8-weeks with or without pretreatment of bovine serum albumin.

Sheikh *et al.,* described that there were areas of haemorrhage within the renal parenchyma, necrosis of renal tubules, infiltration of mononuclear cells, hypercellularity of glomerulus, degenerative prominent nuclei of tubular epithelial cells, narrowing of tubular lumen and certain amount of proteinaceous cast in lumen of convoluted tubules [9]. Taylor in his remarkable study published in American Journal of Pathology described that with the administration of 1.25 mg mercury per kg body weight marked lysosomal changes were induced[10]. Early there was an increase in size and number of droplets per cell. Later the lysosomes appeared in tubular lumens and There was also a reduction in the number and size of droplets long before any changes in histology could be detected.

Many of the alterations were observed primarily in the upper and intermediate sections of proximal convoluted tubules, where there were no histological abnormalities found by conventional staining. Mitochondrial changes consisted of rounding, clumping and apparent increase in enzyme activity. These were more widespread and preceded by 12 hours the appearance of coagulative necrosis in the terminal segments of proximal convoluted tubules. It is likely that the enlargement and the increase in number of lysosomes early in mercury poisoning represented cytolysome formation, and indicated active phagocytosis of damaged cytoplasmic structures.

These changes developed before irreversible mitochondrial and nuclear damage or coagulative necrosis became apparent. Similar changes were reported by Rodin and Crowson while describing nephrotoxicity in rats[11]. Oda and El-Ashmawy reported focal areas of degenerative and necrotic tubular changes, mostly confined to the renal cortex and corticomedullary junction and mild congestion of the intertubular blood vessels and interstitial edema and inflammation in the kidney of rats exposed to Mercury[12].

Jankeer and El-Nouri reported that Renal interstitial haemorrhage associated with expansion of the epithelial layer lining the tubules, necrosis of certain tubules with hyalinization, and infiltration of inflammatory cells in the interstitial tissue developed in the kidneys of Albino mice exposed to another heavy metal i.e. lead (Pb)[13]. Thus, it is deduced that intake of Mercury *kushta* with or without pretreatment of bovine serum albumin was associated with development of major histopathological changes sufficient enough to label nephrotoxicity. However, pretreatment with bovine serum albumin augmented the deleterious nephrotoxic effects of Mercury *kushta* in *Wistar* rats.

## CONCLUSION

An indigenous herbo-mineral preparation (kushta) containing mercury was discovered to have deleterious effects on the kidneys of rats, according to the findings. The kidneys of exposed rats underwent morphological alterations, which included multi-focal and diffuse glomerulonephritis, mesangial widening, increase in glomerular cellularity, thickening of capillary walls and glomerular basement membrane, tubular necrosis, tubular dilatation, tubular vacuolisation, proteineous casts in tubules and mononuclear interstitial inflammation.

However, the histomorphological parameters were more severely affected in the animal groups which were treated with injection of bovine serum albumin as compared to the animal groups who were given Mercury *kushta* without such pretreatment.

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