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ORIGINAL ARTICLE

Histopathological changes due to Gold Kushta in kidneys of Wistar Rats.

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ABSTRACT… Objective: To evaluate the histopathological effects of Gold Kushta in kidneys of Wistar rats. **Study Design:** Experimental study. **Setting:** Department of Pathology, Sahiwal Medical College, Sahiwal. **Period:** 1st of July 2019 till 31st December 2019. **Material & Methods:** The experimental study was conducted on a total of 28 wistar rats of 6 – 8 weeks of age and weighing 200 – 250 grams which were then randomly divided into 4 groups each containing 7 rats. The groups were labelled as G I, II, III and IV. These groups were given preparations of gold in the form of kushta for a period of 8 weeks. The gold kushta was given in the form of pellets. Group I was taken as Control, G II was given single dose kushta 0.15 mg and G III was given double dose kushta 0.3mg on alternate days. Group IV was given a single dose of BSA (bovine serum albumin) 75mg (250mg/kg body wt) at the start of experiment and Gold kushta 0.3 mg on alternate days. Histopathological changes were then seen in mesangium, capillary walls and tubules of kidneys of wistar rats. **Results:** All the parameters were normal in Group 1 while in Groups II, III and IV mesangial proliferation was increased and more diffuse with thickened capillary walls. The cellularity was normal in Group 1 rats but it increased in all the rats of Groups II, III and IV. Mesangial proliferation was greatest in Group IV. None of the rats in Group 1 had epithelial necrosis, dilatation or casts. Epithelial necrosis, dilatation and protein casts were all seen in most of the rats in Groups II, III and IV. **Conclusion:** Thus, gold kushta has detrimental effects over kidneys of wistar rats. These effects are thus increased by concomitant exposure to bovine serum albumin.

Key words: Adenocarcinoma, Cholecystectomy, Cholelithiasis, Demographic, Histopathological, Macroscopic Examination.

INTRODUCTION

Scientifically 'Heavy' metals were only thought to be as metals which have high specific gravity or density. Very recently it was explained that these are actually metals which have the potential to cause human and environmental toxicity[1] and even in low dosages, these can be harmful. Lead, cadmium, arsenic, aluminium, beryllium and mercury are the most common heavy metals that may also cause toxicity above specific dosages.[2]

Even though these heavy metals may cause heavy metal toxicity in even small quantities, some of them are considered essential for human biochemical processes. These are known as essential elements. There are few metals which are used for therapeutic purposes including gold, bismuth, aluminium, lithium and silver.

There is long history of medicinal applications of heavy metals tracing back to 2500 BC such as gold, arsenic and mercury. Nowadays they have even been incorporated in the patent allopathic medications for many diseases for example the use of gold (Au) in chronic rheumatoid arthritis, lithium (Li) in bipolar disorders and manic depression, silver and mercury in microbial infections, arsenic and mercury for the treatment of cancers.[3]

The use of these medicinally important metals is not without any side effects, even when used for a very limited period of time and in small calculated doses which may include nephrotoxicity, hepatotoxicity, carcinomas and hypersensitivity reactions etc.[1]

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In South East Asia the use of this traditional medicine has been in practice since ancient times, Unani/Tibb and Ayurvedic medicine among the most popular systems. In each of these system minerals, herbs and animal tissues are used in various dosage forms. A unique herbo-mineral preparation is, "Kushta", which has long been used by traditional healers. According to their concept preparing a drug in the form of kushta improves safety, potency and efficacy. It may be kept in mind that kushta contains dangerously high quantities of heavy metals. The word 'Kushta' is derived from a Persian word Kushtan meaning "killed or conquered". Gold kushta can be formulated from more than two metal mixtures or alone with metal gold. Gold (Au) metal is present in the form of oxide as a major component of Kushta Tila Kalan in addition to other important herbs. It is mostly used for chronic diseases such as headache, palpitation, arthritis, sexual weakness, asthma, tuberculosis, as a general tonic and immunostimulant.[4] The present study is concerned with these indigenous metallic preparations (Kushtas) of gold and their potential effects on kidneys of wistar rats.

MATERIAL & METHODS

This experimental study was conducted in Department of Pathology, Sahiwal Medical College from 1st of July 2019 till 31st Dec 2019. 28 wistar rats were taken in total which were approximately six weeks age and weighed around 200- 250 grams. After getting approval from the Ethical review committee (24/ME/SLMC/SWL). They were then divided randomly into 4 groups each of which contains seven rats. The groups were labelled as G I, II, III and IV. These groups were given preparations of gold in the form of kushta for a period of eight weeks. The gold kushta was given in the form of small pellets. Group I was taken as Control, G II was given single dose kushta 0.15 mg and G III was given double dose kushta 0.3mg on alternate days. Group IV was given a single dose of BSA (bovine serum albumin) 75mg (250mg/kg body wt) at the start of experiment and Gold kushta 0.3 mg on alternate days. Bovine Serum albumin has the potential to increase permeability of capillaries and cause serum sickness.

At the end of experiment rats were sacrificed. Specimens after being fixed in formal saline were brought to the department of Histopathology, UHS, where these were allocated a specific laboratory number. A detailed gross examination of kidneys was carried out and appropriate sections were taken and paraffin embedded blocks were made. Sections were cut for histopathology examination with Hematoxylin and eosin. Kidneys were also processed for Jones Methanamine silver and Periodic acid schiff stains. Microscopy was done and data was filled in relevant performas.

SPSS (version 20.0) was used for data entry and further analysis. Frequencies and percentages were given for qualitative variables like histopathological changes in kidneys which were analysed using Fisher's exact tests. A p-value of ≤ 0.05 or equivalent was considered as statistically significant.

RESULTS

Histopathological examination of cross sections of kidneys showed that in Group 1, mesangium and capillary walls were normal, while in Groups II, III and IV mesangial proliferation was increased and more diffuse with thickened capillary walls. (Figure-1,2) The cellularity was normal in Group 1 rats but it increased in all the rats of Groups II, III and IV. Mesangial proliferation was greatest in Group IV (Figure-3) (Table-I).

Renal histopathology of renal tubules showed that epithelial necrosis was significantly different in all groups. None of the rats in Group 1 had epithelial necrosis, dilatation or casts. Epithelial necrosis, dilatation and protein casts were all seen in most of the rats in Groups II, III and IV (Figure-4) (Table-II).

DISCUSSION

Clinical manifestations of gold toxicity are allergic reactions, renal tubular and glomerular lesions, bone marrow abnormalities, abnormal liver functions, and skin eruptions. Our study describes the effects of indigenous preparation of gold on kidneys of the rodents.

	Groups (n = %)				P-Value
	Group I	Group II	Group III	Group IV	
Mesangial Proliferation (seg/diffuse)					
None	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
Multifocal	0 (0.0%)	7 (100%)	4 (57.15%)	0 (0.0%)	
Diffuse	0 (100%)	0 (0.0%)	3 (42.85%)	7 (100%)	
Mesangial Proliferation					
None	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
Mild	0 (0.0%)	3 (42.85%)	0 (0.0%)	0 (0.0%)	
Moderate	0 (0.0%)	4 (57.15%)	7 (100%)	3 (42.85%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	4(57.15%)	
Capillary Wall Thickening					
None	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
Mild	0 (0.0%)	4 (57.15%)	4 (57.15%)	3 (42.85%)	
Moderate	0 (0.0%)	3 (42.85%)	3 (42.85%)	4 (57.15%)	
Cellularity					
Normal	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
Increased	0 (0.0%)	7 (100%)	7 (100%)	7 (100%)	

Table-I. Histopathological changes in kidneys of experimental rats

	Groups (n = %)				P-Value
	Group I	Group II	Group III	Group IV	
Epithelial Necrosis					
Absent	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	< 0.001
Present	0 (0.0%)	7 (100%)	7 (100%)	7 (100%)	
Dilatation					
Absent	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.169
Present	0 (0.0%)	4 (57.15%)	3 (42.85%)	4 (57.15%)	
Casts					
Absent	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	< 0.001
Present	0 (0.0%)	7 (100%)	7 (100%)	7 (100%)	

Table-II. Tubular changes in experimental rats

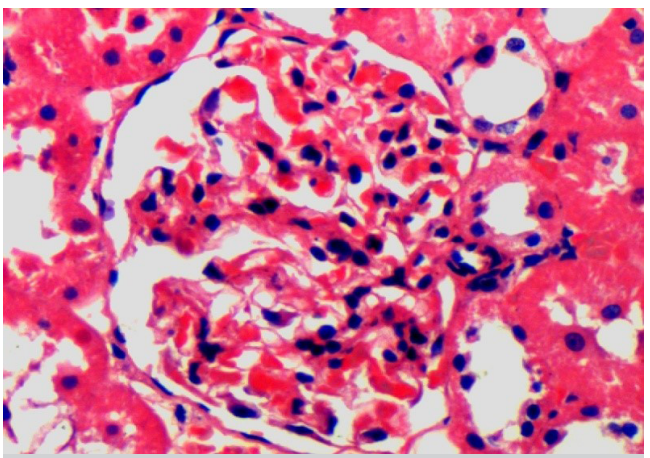

Figure-1. Photomicrograph of kidney in group 2 showing mesangial widening. (H&E, 40x).

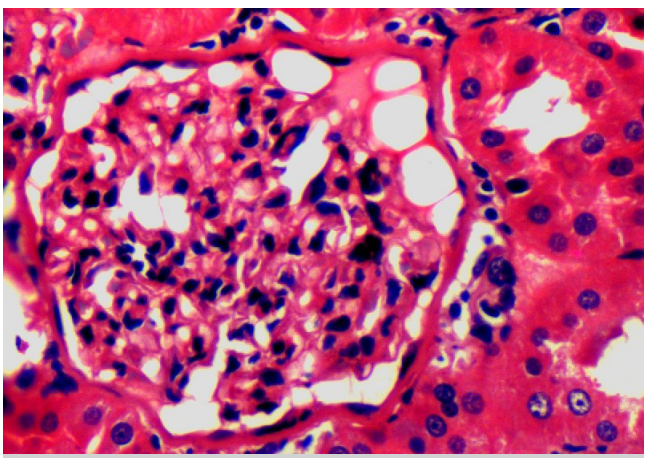

Figure-2. The photomicrograph shows a glomerulus from a kidney of rat in Group 3 having undergone cellular proliferation in a focal manner and multiple capsular adhesions. Capillary wall thickening is also present. (H&E, 40x).

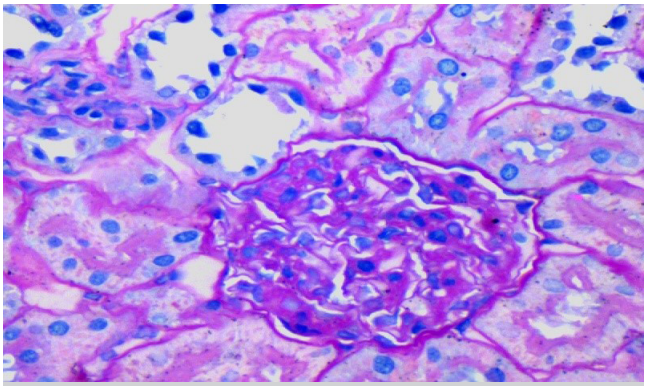

Figure-3. Photomicrograph of a kidney in rat from Group IV shows thickening and widening of mesangium as a prominent feature on PAS. (PAS, 40x)

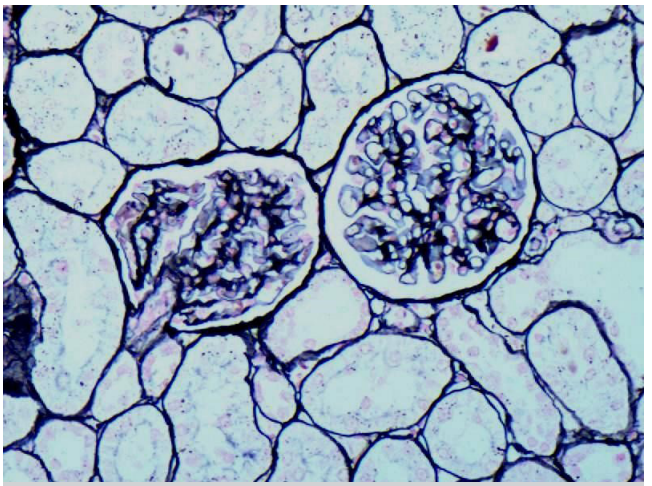

Figure-4. Photomicrograph of kidney glomeruli from an experimental animal of Group IV showing some thickening, widening of mesangium with increase in basement membrane like material, capillary wall is normal. (JMS, 20x)

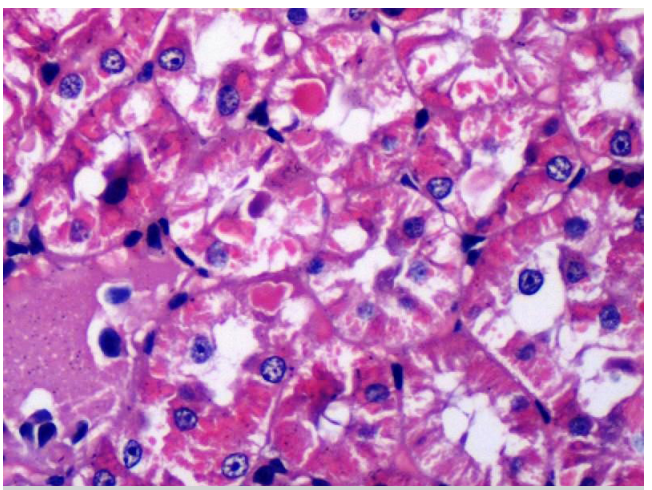

Figure-5. Photomicrograph of proximal convulated tubules of a rat from Group IV showing particles of reabsorbed proteins. (H & E)

Its major effects occur on kidneys as these are basic natural filters and therefore play a vital role in the removal of waste from the body and if they are given in toxic dosages, it can lead to renal failure i.e. nephrotoxicity. Gold therapy leading to renal lesions is well documented in the previous literature.[5] These are mostly glomerular and tubular in nature.[5,6,7,8] The changes in kidneys may vary from slight deformity to marked thickening of the glomerular basement membrane which is related to electron dense deposits 5. Similar changes in glomeruli and renal tubules were demonstrated in 1971 by Nagi and co workers in the experimental study of wistar rats which were treated with gold salts.[8]

In the present experiment both glomerular and tubular changes were seen in the wistar rats, treated by oral traditional preparations. The changes were seen to be more marked in animals of groups G4 which was also introduced with BSA at the start of experiment, thus inducing acute serum sickness phase. These findings indicated that oral preparations of gold (kushta) can surely produce renal damage including necrosis in renal tubules and immune complex nephritis.

Gold particles cause toxic effect to the proximal convulated tubules. The epithelial cells are degenerated and at other places there is sloughing of epithelial cells. Tubular necrosis is observed in almost all cases. Tubules are filled with protein casts. We also observed reabsorbed protein particles in the tubular epithelial cells. Gold is a well known toxin for proximal tubules in kidneys, where it gets localized to mitrochondria of the cells in both acutely and chronically intoxicated rats, resulting in necrosis of tubules. The characterstic feature of gold induced membranous glomerulopathy is the demonstration of gold inclusion forming electron dense filamentous strands in proximal tubular epithelial cells, mesangial cells and glomerular epithelial cells.[9]

In glomeruli mesangial proliferation is observed which varies in intensity in different groups. In low doses it is multifocal in distribution while in higher doses there is diffuse proliferation. Both mesangial

matrix and mesangial cells proliferate. All these were also seen in the study done by Kapoor et al in 2010.[10] Findings in glomeruli are very much similar to idiopathic membranous nephropathy along with typical immune complexes in the form of electron dense deposits which are identified in the glomeruli on electron microscopy as seen by Katz et al.[11] Lasagna-Reeves et al closely investigated nephropathy related to gold in his experimental rats which was induced by sodium aurothiomalate and also showed that in lower doses kidney damage is due to the toxic action of gold directly on proximal convoluted tubules and in high doses it is due to the stimulation of immune system.[12] Crescent formation was observed in one case. One of the renal lesion (sclerosis) is not observed in any of our rats however Nagamato et al and Norn et al reported it in their experimental study on wistar rats with a weekly injection of sodium aurothiomalate for a period of 3 months. The pathological process is thought to be similar as in human beings.[13,14]

CONCLUSION

Thus, gold kushta has detrimental effects over kidneys of wistar rats. These effects are thus increased by concomitant exposure to bovine serum albumin.

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No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Saadia Raza	Collection of data, Conception and design.	
2	Sadaf Shafique	Manuscript writing.	
3	Raees Abbas Lail	Editing and review of manuscript.	
4	Syed Saad Gardezi	Statistical analysis.	
5	Sakina Jamil	Drafting and Critical reviewing.	
6	A.H. Nagi	Analysis of data.	