

## BIOCHEMICAL CHANGES IN GOLD KUSHTA INDUCED NEPHROTOXICITY IN RATS

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### ABSTRACT

**Background and Objectives:** This experimental study was designed to evaluate nephrotoxicity of indigenous gold preparations (Kushtas) in Wistar rats. There were biochemical changes observed in the rodents resulting from renal damage, after the administration of indigenous gold preparations.

**Methodology:** The study was conducted in a total of 28 healthy Wistar rats, each weighing 200 – 250 grams. The rats were randomly divided into 4 groups each containing 7 rats. Group I was taken as control, Group II was given single dose (0.15 mg) of gold kushta, Group III received double dose (0.3 mg) kushta, and Group IV double dose kushta (0.3 mg) along with BSA (Bovine Serum Albumin) 75 mg. Biochemical parameters included spot urinary proteins and serum creatinine.

**Results:** The changes were observed in all the above groups except group G I which served as control. More severe changes were seen in high dose groups and the group given BSA injection alongwith kushta (Group III, IV).

**Conclusions:** This experiment proves that the herbomineral preparations (kushta) of gold which are nephrotoxic in rats may have similar toxic effects in human beings.

**Key Words:** Heavy metals, Kushta nephrotoxicity, Proteinuria, Serum creatinine.

### INTRODUCTION

Scientifically, 'heavy' metals are described as metals having high density or high specific gravity; however, recently they have been defined as metals having potential human and environmental toxicity.<sup>1-3</sup> They are toxic even at low concentrations. The most common heavy metals involved (implicated) in human toxicity include lead, mercury, cadmium and arsenic, although beryllium, aluminium, manganese and cobalt may also cause toxicity.<sup>4</sup> Heavy metals have a long history of medicinal application, as medical use of gold, arsenic and mercury can be traced back to the 2500 BC. They are even used now a days in the allopathic patent medications for different diseases e.g; gold (Au) in chronic rheumatoid arthritis, platinum and arsenic (As) in cancers, lithium (Li) in manic depression, silver and mercury in microbial infections.<sup>5</sup>

Heavy metals disrupt metabolic functions in two ways:

1. Accumulate in vital organs and glands such as heart, brain, kidneys, liver, bone, etc and thereby disrupt function.
2. They also displace the vital nutritional minerals from their original place, and thus hindering their biological function.<sup>6</sup>

Although used in very small calculated doses for limited time period and under special circumstances

they are not without side effects and induce hypersensitivity reactions, hepatotoxicity, nephrotoxicity, carcinomas etc.<sup>1</sup>

In addition to the conventional medication, the traditional or indigenous medicine has long been practiced in the South East Asia, Ayurvedic and Unani / Tibb are examples of the two most popular systems. Each of the systems use herbs, minerals and animal tissues in various dosage forms. "KUSHTA" (derived from KUSHTAN a Persian word meaning "to kill") is the unique herbo-mineral preparation, which has long been used by traditional healers. Kushta contains dangerously high quantities of heavy metals. They are well known for their quick and prompt effect even in small doses.<sup>7</sup> The adverse effects of kushtas are due to the binding of heavy metals to oxygen, nitrogen and sulfhydryl group in proteins resulting in alteration of enzymatic activity (Soghoian, and Sinert, 2009). In addition the metal interacts with DNA resulting in cross links between DNA strands and metal.<sup>8</sup> Kushta is prepared by constant heating of the metal for many times with herbal juices in a sealed claypot after which wet grinding is done which converts metal into very fine particles which are easily ingestible.<sup>2</sup> As a result of constant heating metal is converted into oxide form that is active and adversely affects nearly every system of body including haematopoietic, renal, cardiovas-

cular (CVS), gastrointestinal (GIT), central nervous system (CNS) and even integumentary system. According to the traditional healers this constant heating kill all the harmful effects of the metal.<sup>2,3</sup> After absorption from gastrointestinal tract it binds to albumin and then distributed to various tissues including macrophages, erythrocytes, kidney, liver, spleen, lymph nodes, bone marrow, adrenal glands, testes and skin. About when 60 – 90% of gold excretion is through kidneys and the remaining 10 – 40% via biliary excretion into the faeces.<sup>1,9</sup> When given in the form of traditional or indigenous preparations gold shows immunostimulant activity on macrophage functions in contrast to the immunosuppressive effects of AN.<sup>10,11</sup> Renal lesions associated with the use of organic salts of gold to treat rheumatoid arthritis are well documented.<sup>12-16</sup> The main sites of deposition of the gold salts are epithelial cells of proximal convoluted tubules initially and later on in small amounts in distal tubules, interstitial tissue and glomeruli. The adverse effects include proteinuria, glomerulitis with haematuria, Membranous glomerulonephritis, Nephrotic syndrome morphologically consists of an immune – complex glomerulonephritis, with granular deposits along the glomerular basement membrane and in the mesangium.<sup>17,18</sup>

The present study is concerned with indigenous metallic preparations (Kushtas) of gold. Gold kushta can be made with gold alone or may be a mixture of more than two metals. Kushta tila kalan, contains gold (Au) in oxide form as a major component along with other herbs is used for arthritis, chronic headache, palpitation, asthma, tuberculosis, sexual weakness, as a general tonic and immunostimulant.<sup>19,2</sup>

**METHODOLOGY**

This experimental study was conducted in Department of Pathology, University of Health Sciences, Lahore. A total of 28 Wistar rats of 6 – 8 weeks of age and weighing 200 – 250 grams were procured from University of Veterinary and Animal Sciences, Lahore. They were randomly divided into 4 groups each containing 7 rats. The groups were labeled as G I, II, III and IV. These groups were given gold kushta for a period of 8 weeks. The gold kushta was given in the form of pellets. Gro-

**Table 1:** Biochemical parameters including proteinuria and serum creatinine in all 6 groups. The minimum, maximum and mean values along with their P-values (which are between the groups) are given. The proteinuria and serum creatinine level shows serial rise.

Parameters	GI	GII	GIII	GIV	P-value
Proteinuria					
Start					
Mean	0	0	0	0	0.000
Range	0	0	0	0	
Mild					
Mean	0	261.43	185.71	175.71	0.001
Range	0	30 – 500	100 – 500	30 – 500	
End					
Mean	0	371.43	414.29	442.86	0.000
Range	0	200 – 500	300 – 500	300 – 500	
Serum Creatinine					
Start	0.785	0.814	0.871	0.73	0.140
Range	0.6 – 1	0.7 – 1.0	0.7 – 1.0	0.65 – 0.84	
Mild					
Mean	0.757	1.35	1.38	1.79	0.000
Range	0.6 – 0.9	0.8 – 1.9	1.13 – 1.71	1.50 – 1.98	
End					
Mean	0.757	2.05	2.28	2.52	0.000
Range	0.6 – 1.0	1.73 – 2.44	1.94 – 2.53	2.16 – 2.80	

up I was taken as Control, G II was given single dose kushta 0.15 mg daily, G III was given double dose kushta 0.3 mg on alternate days, G IV was given double dose kushta on alternate days with a single dose of BSA (Bovine Serum Albumin) 75 mg (250 mg/Kg body wt). It was given at the start of experiment. BSA causes serum sickness and increases capillary permeability. Biochemical parameters proteinuria and serum creatinine were done at the start, mid and end of the experiment. Medi-test Combi 8, urinary strips were used to assess proteinuria. Serum creatinine was estimated by Randox Kits. The results were entered in the relevant proforma.

**RESULTS**

In this experiment, biochemical changes were observed in the rodents due to damage in the kidney. The proteinuria was nil at the start of the experiment which

then showed a gradual rise till 8 weeks, where it reached a maximum level at the end of the experiment. Same was the case with serum creatinine, at the start of the experiment it was within normal range and then it showed a progressive rise (Table 1). Group I which was control group showed nil proteinuria and normal limits of serum creatinine throughout the experiment. In Group II, the proteinuria was not seen at the start of the experiment. However it appeared in significant amounts in the middle of the experiment (at 4 week) and it rose to variable but higher levels i.e. 500 mg/dl by the end of experiment. As regards serum creatinine levels most of the animals showed increasing levels of serum creatinine which too showed a rising pattern after week 1 to week 8 (range 1.03 – 2.31 mg/dl). In Group III, a rising pattern from week 1 to week 8 (range 1.13 – 2.53 mg/dl) was observed in the levels of serum creatinine of most of the animals. Similarly in Group IV serum creatinine levels showed rising pattern from week 1 – 8, (range 1.50 – 2.80 mg/dl).

## DISCUSSION

Kidneys being the natural filters bear the major burnt of change effectors. They play an essential role in removing the debris and if they are highly toxic can lead to renal failure (nephrotoxicity). Renal lesions associated with the gold therapy is well documented. These are tubular and glomerular in nature.<sup>21,15,20</sup> The renal changes vary from slight irregularity to marked thickening of the glomerular basement membrane which is associated with electron dense deposits.<sup>20</sup> On the other hand Nagi and coworkers demonstrated similar renal tubular and glomerular changes in the experimental model of Wistar rats treated with gold salts.<sup>15,2</sup>

The present study describes the effects of indigenous gold preparation on the kidney of the rodents. In this study significant elevation was observed in the levels of serum creatinine, and total urinary proteins which are the presumptive markers of gold nephropathy. In the experimental groups (G II, G III, G IV) elevated levels of these parameters in serum and urine were observed.

The continuous accumulation of gold in the liver, spleen and kidney destroy the immune system and thus causes the irreparable damage to these organs. The kidneys cannot get their protection by the immune system. Finally, the haematologic and biochemical changes reflect these damages.<sup>22</sup> Proteinuria being the indicator of renal damage showed continuous rise in its levels. Similar results were found by Nagi and coworkers in two different experiments of gold induced nephropathy conducted on rats and rabbits respectively.<sup>15,20</sup> Another experimental study of gold induced nephropathy in guinea pigs by sodium aurothiomalate favours our results. Proteinuria was recorded as nil in the start of experiment in all the groups and then it showed a progressive rise in all groups except in group

I, which is control. The rise was more in the groups G III (double dose) and G IV (in which BSA induced serum sickness was produced).

The Creatinine is the other important indicator of renal function.<sup>22</sup> It also showed a serial rise in creatinine in the experimental animals. The experimental model produced by Nagi, et al, favours our results. These effects are due to both tubular and glomerular damage in the Wistar rats, treated by oral indigenous preparations. Proximal convoluted tubules are damaged by the toxic effect of gold particles. Gold is already known to be toxic to proximal tubules, where it localises to the mitochondria of the cells in acutely and chronically intoxicated rats, resulting in tubular necrosis, similar to idiopathic membranous nephropathy.<sup>23,24</sup>

Nagi, et al, investigated gold nephropathy in experimental rats induced by sodium aurothiomalate and showed that in higher doses renal damage is due to the direct toxic action of gold on proximal convoluted tubules and in lower doses due to the stimulation of immune system.<sup>1,2</sup>

It is **concluded** that herbomineral preparations of gold (kushtas) are definitely nephrotoxic and can be responsible for renal failure of varying degrees of severity depending upon the amount and duration for which these substances are used in human beings.

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