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ORIGINAL ARTICLE

Morphological changes in liver of wistar rats induced by Gold Kushta.

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ABSTRACT… Objective: To observe hepatic changes induced by Gold Kushta. In our study the histopathological effects of Gold Kushta was seen in livers of Wistar rats. **Study Design:** Experimental study. **Setting:** Department of Pathology, Sahiwal Medical College, Sahiwal. **Period:** 1st July 2019 to 31st Dec 2019. **Material & Method:** 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 lobules, sinusoids, kupffer cells and central veins of livers of wistar rats. **Results:** The histological examination of liver showed sinusoidal congestion and central vein congestion, apoptosis, necrosis and hydropic degeneration as common features. In addition fatty degeneration, inflammation and kupffer cell hyperplasia were also seen in few cases. These changes were observed in all the above groups except group G I serving as control. More changes were seen in high dose groups and the groups given BSA inj. (Group II, III and IV). **Conclusion:** Thus, indigenous preparations of gold kushta have detrimental effects over livers of wistar rats and are thus hepatotoxic.

Key words: Congestion, Carcinomas, Degeneration, Histopathological, Hepatotoxicity, Hyperplasia, Nephrotoxicity.

INTRODUCTION:

Heavy metals, as described scientifically, have higher specific gravity. They have the potential to cause toxicity in humans and environment.[1,2] Mercury, arsenic, cadmium, lead, beryllium, aluminium, cobalt and manganese are the most common heavymetals enlisted to cause human toxicity.[3,4]

Those elements which are considered as heavy metals have known to have no beneficial effect on human physiology and are termed as toxic metals. The few important examples are cadmium, mercury and lead. There are also other metals which are considered very important for biochemical processes in humans. They are also known as essential elements. There are many such examples such as iron in hemoglobin, zinc being a cofactor for various enzymatic reactions in body and cobalt forming core of vitamin B-12. Similarly, there are trace elements which include molybdenum, selenium, copper and manganese which are important for human diet although being trace elements. There is another metal group which is famous in therapeutic medicine such as lithium, gold, silver, aluminium, gallium and bismuth.

There is a long history dating back to 2500 BC regarding the medicinal use of gold, arsenic and mercury. Today they are being used in allopathic medications for many different diseases such as; gold (Au) in chronic rheumatoid arthritis, silver and mercury in microbial infections, lithium (Li) in manic depression , platinum and arsenic (As) in malignant tumors.[5]

In the region of South East Asia, traditional

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medication has been practiced since long ago in addition to the conventional medication. The two most popular systems are Ayurvedic and Unani/ Tibb medicine. In each of these systems, there is calculated usage of herbs, minerals and animal tissues in various dosages. The most famous and unique herbo-mineral preparation being used by traditional healers is named as "KUSHTA". Kushta preparations are well known for their quick and prompt effect even in small doses.[6]

The preparation of Kushta is itself unique and requires expertise as well. It is prepared by the constant heating of the metal with herbal juices for several times. This process is done in a sealed clay pot. After heating it for a calculated amount of time, it is grinded well to convert it into very fine small particles which can easily be digested.[7] During this whole procedure, constant heating is done which converts the metal into its respective oxide form. This oxide form is active in nature and thus every bodily system gets adversely affected which include the vital cardiovascular, gastrointestinal, hematopoietic, renal and central nervous system.[2,8]

The pathogenesis behind these heavy metals causing adverse effects in the form of kushtas are due to their binding to atoms oxygen, nitrogen and sulfhydryl group in proteins on cellular and molecular level resulting in hampering of enzymatic activity.[2] In addition to this, cross linkages may also occur between the DNA strands and metal causing damage to nuclear material.[9]

As liver is a vital organ it is a prime target for heavy metal toxicity. Gold particles in the form of kushta if not given in calculated amounts can be very toxic, producing hepatitis, cholestasis and may also be related to diseases causing jaundice. These can be reversible if small dosages have been taken for a brief period of time but however severity may increase with increasing dose and duration. The toxicity can be a result of idiosyncratic reaction. There can be uncontrolled release of inflammatory mediators and reactive oxygen species due to the activation of Kupffer cells enhancing hepatocytic injury.[10,11]

Our present study is regarding the potential effects on liver of wistar rats with indigenous metallic preparations (Kushtas) of gold.

MATERIAL & METHODS

The experimental study was conducted in Department of Pathology, Sahiwal Medical College from 1st of July 2019 till 31st Dec 2019 after approval of the ethical review committee (No.24/ME/SLMC/SWL). Twenty eight wistar rats of approximately age 6 to 8 weeks and having weight between approx. 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 labelled as G I, II, III, IV, V and VI. 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. Serum sickness was caused by BSA and capillary permeability also gets increased. At the end of experiment all the rats were sacrificed. Specimens were fixed in formal saline and then they were brought to the department of Histopathology, UHS, where these were given a specific laboratory number. A detailed gross examination of Livers was carried out and appropriate sections were taken and paraffin embedded blocks were made. Sections were cut for histopathology examination with Hematoxylin and eosin. Livers were also processed for Periodic acid schiff and reticulin stains. Microscopy was done and data was filled in relevant performas.

SPSS of version 20.0 was used for data entry and its 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

In Group 1, morphological analysis of liver showed normal lobular architecture of the liver. In Group II and Group III, only focal effacement was seen in 7(100%) and 4 (57.15%) of the rats respectively whereas marked lobular effacement was seen in 7(100%) of the rats in Group IV. 3 (42.85%) animals showed moderate apoptosis and 4(57.14%) showed mild apoptosis, while in Groups III and IV there was moderate apoptosis present in 7 (100%) and 3 (42.85%), respectively. Sinusoidal congestion was seen in only 1(14.28%) of the animals of Group II while in 3 (42.85%) of the animals in both the Groups III and IV (Figure-3). 5 (71.43%) animals of Group II, 4 (57.15%) of Group III but only 2 (28.57%) of the animals of Group IV showed kupffer cell hyperplasia (Figure-1). Only mild necrosis was present in 4 (57.15%) animals of Groups III and IV but moderate necrosis in 4 (57.15%) of the rats where as inflammation was seen in most of the animals of Groups II (85.72%), III and IV (71.43%) (Figure-2) (Table-I).

DISCUSSION

One of the many clinical manifestations of gold toxicity include allergic reactions, bone marrow abnormalities, skin eruptions, abnormal liver functions, lesions of glomeruli and renal tubules. In the present study, the effects of patent and indigenous gold preparations are seen in the liver of wistar rats.

	Groups (n = %)				
	Group I	**Group II**	**Group III**	**Group IV**	**P-Value**
Lobules					
Normal	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
Focal effacement	0 (0.0%)	7 (100%)	4 (57.15%)	0 (0.0%)	
Effacement	0 (100%)	0 (0.0%)	3 (42.85%)	7 (100%)	
Apoptosis					
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%)	
Necrosis					
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%)	
Hydropic Degeneration					
Absent	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
Present	0 (0.0%)	7 (100%)	7 (100%)	7 (100%)	
Central Vein Congestion					
Absent	7 (100%)	5 (71.43%)	4 (57.15%)	4 (57.15%)	0.101
Present	0 (0.0%)	2 (28.57%)	3 (42.85%)	3 (42.85%)	
Sinusoidal Congestion					
Absent	7 (100%)	6 (85.72%)	4 (57.15%)	4 (57.15%)	0.056
Present	0 (0.0%)	1 (14.28%)	3 (42.85%)	3 (42.85%)	
Kupffer Cell Hyperplasia					
Absent	7 (100%)	2 (28.57%)	3 (42.85%)	5 (71.43%)	0.047
Present	0 (0.0%)	5 (71.43%)	4 (57.15%)	2 (28.57%)	
Inflammation					
Absent	7 (100%)	1 (14.28%)	2 (28.57%)	2 (28.57%)	0.339
Present	0 (0.0%)	6 (85.72%)	5 (71.43%)	5 (71.43%)	
Fatty Degeneration					
Absent	7 (100%)	7 (100%)	1 (14.28%)	3 (42.85%)	0.134
Present	0 (0.0%)	0 (0.0%)	6 (85.72%)	4 (57.15%)	

Table-I

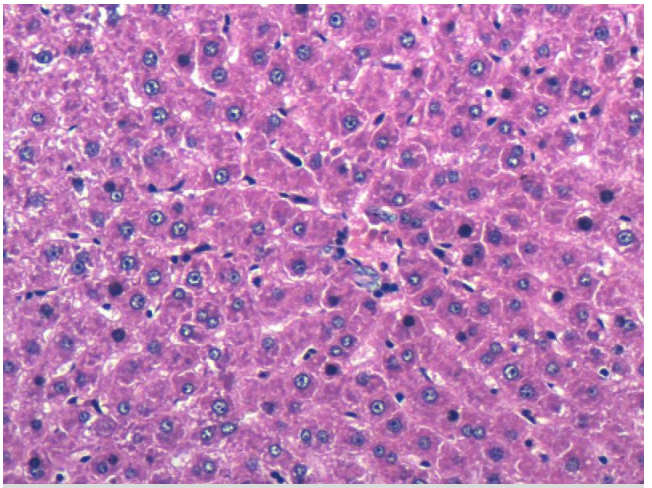

Figure-1. Photomicrograph of liver tissue of rat showing kupffer cell hyperplasia. (H&E, 20x)

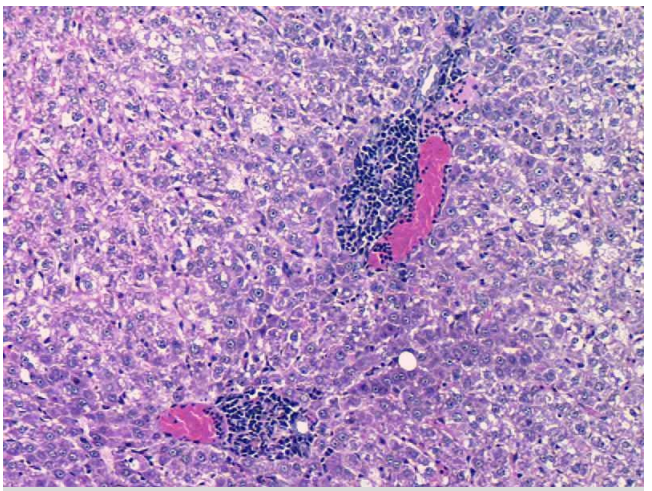

Figure-2. Photomicrograph of liver lobule showing chronic inflammatory infiltrate around central vein, (H&E, 10x)

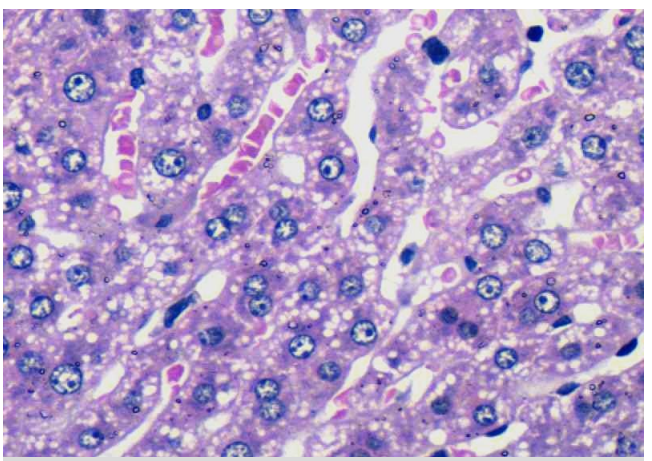

Figure-3. Photomicrograph of liver tissue showing sinusoidal congestion within the hepatocytes. (PAS, 40x)

The immune system of body gets destroyed by the continuous accumulation of gold in vital organs of the body including kidneys, spleen and liver and hence causing irreversible damage to them.[12]

On microscopical examination, according to our study, there were evident changes seen in the morphology of gold treated livers of rats as compared to control group. In gold treated rats, many architectural changes have been observed in the liver tissue such as lobular; these changes were more evident in the gold treated rats given double dose of gold kushta with BSA.[13] There was mild apoptosis seen in rats treated with a single dose of kushta whereas moderate apoptosis in almost all the rats present in groups treated with double dose of kushta alone and with BSA. Apoptosis is defined as a type of natural cell death and needs to be overcome when uncontrolled. There is resorption of cells in an orderly manner during apoptosis with minimal leakage of cellular debris and inflammation. Each dying cell also gets separated from the neighbouring cells.[14]

There are other findings also seen in the rats given high dosage which include central vein congestion, sinusoidal congestion and dilatation. Vessel walls may also get damaged in some cases. These same changes have also been observed in other heavy metal toxicities.[15]

Although present less frequently, but fatty change is also seen in our study. It is observed that abnormal and impaired fat metabolism occurs due to damage to the rough endoplasmic reticulum and detachment of cytoplasmic lipoproteins secondary to lipid peroxidation. This further indicates that there is abnormal retention of lipids in the hepatocytes due to toxic damage done by gold particles. In portal and periportal areas, there is inflammatory cell infiltration. This phenomenon suggests that the gold metal particles cause interference in the anti oxidant defense mechanisms of hepatic interstitial tissue by interacting with proteins and enzyme systems. This then leads to initiation of an inflammatory response by generating reactive oxygen species. In hepatocytes, Kupffer cell hyperplasia

is seen showing the defense mechanism of detoxification. There is increase in phagocytic activity of sinusoidal cells playing an important role in removing gold particles by increase in the number of kupffer cells.[13]

The experimental rats in our study present in high dosage groups show sporadic necrosis which is most likely to be due to the oxidative stress secondary to glutathione depletion.

The histological findings, as explained above, may be the result of gold particles interfering with the antioxidant defense mechanism inducing high stress in hepatocytes which in the end get necrosed or atrophied.

Our current study confirms our hypothesis that the above mentioned herbomineral preparations (kushta) of gold are toxic to liver which is dose related and also depends upon the duration of the usage. The underlying mechanism is thought to be immune mediated, however it requires further studies at cellular and molecular levels.

CONCLUSION

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

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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